Ecological consequences of angiosperm genome size and macronutrient availability

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Statement of originality

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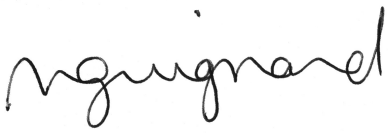
Signature 

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Details of collaboration and publications are specified at the beginning of each chapter where applicable.

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Abstract

Genome size (GS) is a fundamental trait influencing cellular, developmental and ecological parameters, and varies c. 2400- fold in angiosperms. This astonishing range has the potential to influence a plant's nutrient demands, since nucleic acids are amongst the most phosphate and nitrogen demanding cellular biomolecules, and hence its ability to grow and compete in environments where macronutrients are limited. Angiosperm GS are strongly skewed towards small genomes, despite the prevalence of polyploidy in the ancestry of most if not all angiosperm lineages.

This thesis examines the hypothesis that large genome sizes are costly to build and maintain and that angiosperm species with large GS are constrained by nitrogen and phosphate limitation. It untangles the interactions between GS, polyploidy and competition in plant communities, and examines how herbivory and GS play a role in plant productivity, measured as above-ground biomass.

The hypothesis that large GS are costly was approached by analysing: 1) plant communities growing under different macronutrient conditions at the Park Grass Experiment (Rothamsted, UK); 2) plant communities under different conditions of macronutrient limitation and insect, mollusc, and rabbit herbivory at Nash's Field in Silwood Park (UK); and, 3) Ellenberg's indicator values which represent the realised niche of a species in terms light, water, and soil fertility.

Support for the hypothesis was found in all experiments. The range of analyses show that angiosperm plants with large genomes (e.g. 1C-value > 5 pg) are indeed under greater macronutrient limitation in comparison to plants with small genomes, and that it is polyploid plants with large GS which are the most competitive when macronutrient resources are plentiful. In terms of herbivory, the key finding is a highly significant

negative association between GS and rabbit herbivory. A species' realised niche for soil fertility was found to show a positive association with its GS. Overall the thesis shows that angiosperm GS plays a central role in plant community composition and responses to macronutrient conditions, and potentially on higher ecosystem processes through associations at different trophic levels.

Contents

Each Appendix begins with a table of contents.

List of figures

List of tables

List of appendices

Appendix 1

Appendix 2:

Appendix 3:

Chapter 1 General Introduction

Genome size in flowering plants

Angiosperm genome sizes (GS) range c. 2400-fold, from a 1C-value of 0.059pg (61Mbp) in *Genlisea tuberosa* (Lentibulariaceae) (Fleischmann *et al.*, 2014), to 152.23 pg in *Paris japonica* (Melanthiaceae), the largest known eukaryote genome (Pellicer *et al.*, 2010). The 1C-value (holoploid) refers to the amount of DNA in a gametic nucleus and is measured in picograms (pg) (1 pg = 978 megabase pairs), where C stands for "nothing more glamorous than constant" (Swift, 1950; Bennett & Leitch, 2005). Despite such a large range, angiosperm GS are skewed towards small values (mean 1C-value = 5.48 pg, median = 1.93 pg, n = 8881) (Bennett & Leitch, 2012, plus additional values not yet published) (Fig. 1.1).

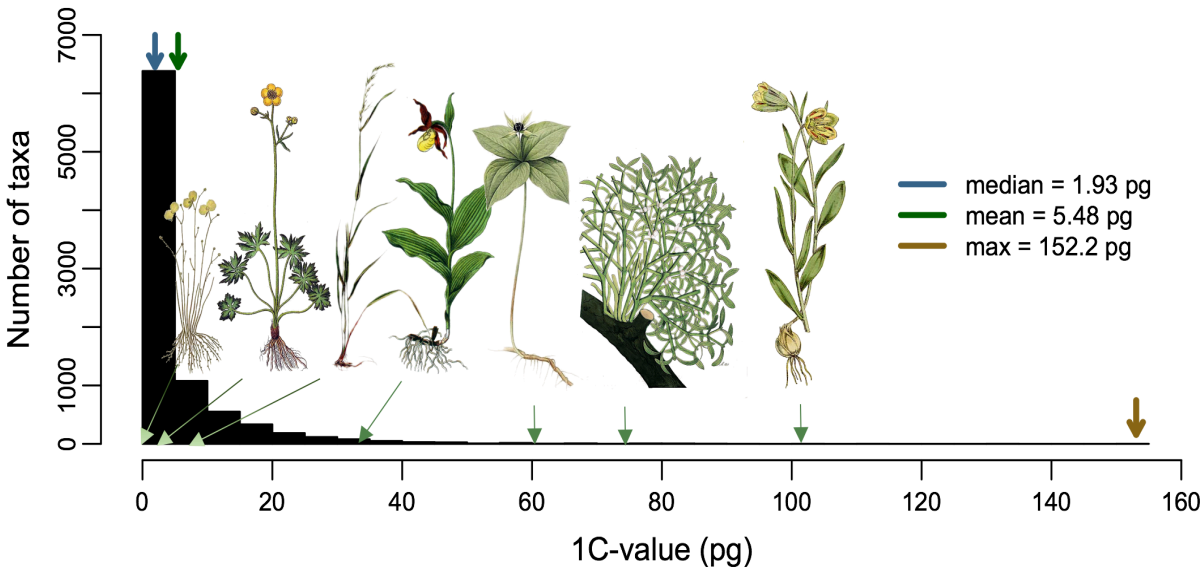

Figure 1. 1 The distribution in angiosperm genome sizes is highly skewed towards small values. Shown, from left to right is: *Utricularia gibba* (Lentibulariaceae), 1C = 0.09; *Ranunculus acris* (Ranunculaceae) 1C = 4.5 in diploid, 1C = 7.8 in tetraploid; *Arrhenatherum elatius* (Poaceae), 1C = 8.0; *Cypripedium calceolus* (Orchidaceae), 1C = 32.5; *Paris quadrifolia* (Melanthiaceae), 1C = 60.1; *Viscum album* (Santalaceae), 1C = 76.0; *Fritillaria elwesii* (Liliaceae), 1C = 103.65 pg. *U. gibba* and *F. elwesii* are the fifth-smallest, and fourth-largest known angiosperm genome sizes respectively.

The skew towards small C-values in angiosperms is not observed in other vascular plant groups (Leitch & Leitch, 2013) and may be a reflection of costs and limitations associated with a large genome for fast growing plant species. Investigations have revealed a suite of relationships between GS and phenotype (e.g. increased cell size), ecological distributions, and evolutionary processes, which point towards GS being a trait upon which natural selection can act (Greilhuber & Leitch, 2013). Indeed, the nucleotype hypothesis (Bennett, 1972) proposes that, in addition to the information encoded in DNA, the amount of DNA itself (the bulk DNA) has an effect on phenotype (discussed further below).

There has been debate relating to the relative influence of selective versus stochastic processes in the divergence of genome size (GS) (reviewed in Gregory, 2001). It has been argued that GS has a non-adaptive role and the large diversity of GSs have arisen from neutral processes, such as the differential rates of DNA insertions and deletions (Petrov, 2002) and that rates of change are proportional to GS (Oliver *et al.*, 2007), but see Gregory (2004). In considering evolutionary processes influencing GS, the reproductive system (Whitney *et al.*, 2011) and effective population sizes (Lynch & Conery, 2003; Lynch, 2011) need to be considered, especially the latter, where the strength of genetic drift can outweigh the strength of selection in small populations. But there remains little consensus as to the importance of stochastic processes, and selection on GS may operate at different levels (nuclear, cellular, tissue or organismal) over long time frames. The environment certainly has the potential to play a selective role on GS, as it imposes abiotic selection pressures in the shape of resource limitations and climatic stress, and biotic pressures from the need to compete with other organisms and tolerate predation and attack.

Does selection act on GS?

Several hypotheses have been proposed to explain how selection might act on GS, with large GS being selected against in certain circumstances, which together are encapsulated in the "large genome constraint hypothesis" (Knight *et al.*, 2005). These hypotheses include:

1. Potentially, rapid growth rates, especially under nutrient-limited conditions, would be predicted to select for a smaller, more streamlined genome (Hessen *et al.*, 2010). This might arise because high growth rates result in a trade-off between the allocation of phosphate (P) and nitrogen (N) to DNA or RNA, which would impose selection pressures on taxa with larger, more 'N and P costly' genomes. This hypothesis, and the importance of N and P is investigated in detail in this thesis, and is discussed in more detail below.
2. Cavalier-Smith (Cavalier-Smith, 2005) proposed that natural selection may act against a large GS because of its influence on cell volumes and cell cycles (nucleoskeletal theory). This theory emphasizes an optimal ratio of the nuclear to cell volume, where GS is an outcome of selection at the cell level for economy and rapid reproduction (smaller genomes), and the balance for maintaining enough DNA as a scaffolding element.
3. A larger GS is correlated with an increase in guard cell length and decrease in stomata density (Greilhuber & Leitch, 2013). These phenotypes may provide a selection pressure against large GSs arising from less efficient stomatal conductance and lower rates of gas exchange, in part due to longer diffusion paths (Franks *et al.*, 2009; Drake *et al.*, 2013). This in turn may impose limits on the type of habitat a taxon may occupy, since for example, species with larger stomata are more drought sensitive (Knight & Beaulieu, 2008; Carta & Peruzzi, 2016) and respond more slowly to water stress than species with smaller stomata.
4. One of the most profound effects of GS may be on the duration of mitosis and meiosis, which increase with GS (Fig. 1.2). Large genomes require a longer time for DNA

replication (with an average rate of 0.3 hours per pg of DNA (van't Hof, 1965)) and for DNA to decondense (Gregory, 2001). Thus GS imposes a minimum generation time (parent-to-parent) (Bennett, 1972), which, in turn, can impose limitations on "where, when and how plants [and animals] grow" (Bennett, 1987). For example, for a plant to have a short life cycle (i.e. an annual) it "requires" a small GS because of the time needed to undergo a sufficient number of cell divisions to grow quickly enough. Above a certain threshold of GS, estimated at 1C= c. 25 pg, the increase in the duration of the somatic cell cycle means plants become obligate perennials (Bennett, 1972) (e.g. *Fritillaria elwesii*) (Fig. 1.2, 1.3). A meta-analysis comparing life histories with

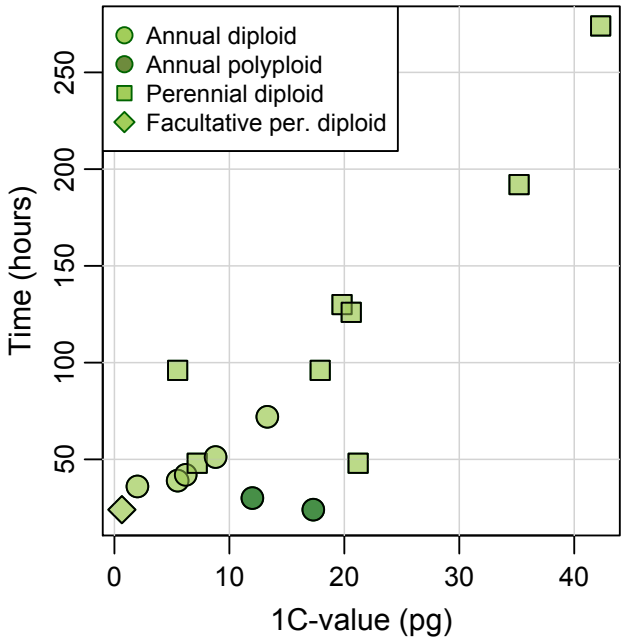

Figure 1. 2 Relationship between the duration of meiosis and 1C-value. Length of time ranges from 24 hours in *Antirrhinum majus* (Plantaginaceae) (1C-value = 0.65 pg), to 274 hours in *Trillium erectum* (Melanthiaceae)(1C-value = 42.3pg). Duration of meiosis and ploidy levels are obtained from Bennett 1972, Table 2, and the GS values are taken from the Plant DNA C-values database (Bennett & Leitch, 2012). One species, *A. majus* is a facultative perennial, i.e. sets seed in the first year. Plants with a very large GS become obligate perennials (Bennett, 1972) (e.g. *Fritillaria elwesii*).

duration of the cell cycle (Bennett, 1972) showed that annuals completed their cell cycle times in around 12 h (n = 19), versus 21.8 h in perennial species (n=8). Similarly, the duration of meiosis is shorter in annuals (39.2 h in 9 species) than in perennials (133.9 h in 13 species) (Bennett, 1972). Ephemeral plants, which complete their life cycle within a few months were found to have an even narrower range of small GSs than annuals (1C-value < 3 pg). However, taxa with small genomes are not limited by GS in the diversity of life histories they can adopt, and are represented in all life history types, from ephemeral plants such as *Cardamine hirsuta* (Brassicaceae), 1C= 0.2 pg, and perennial evergreen herbaceous species such as *Phormium tenax* (Asphodelaceae), 1C= 0.76 pg, to evergreen trees (e.g. *Pyrus calleryana* (Rosaceae), 1C= 0.6pg).

Genome size has also been shown to be associated with other plant characteristics and growth forms. The duration of fertilisation, which is the time beginning with the penetration of the embryo sac by male gametophytes to their fusion with female nuclei, correlates with GS, ranging from 'immediate' in species with a GS less than 12 pg (e.g. *Crepis capillaris*, *Pisum sativum*) to over three days in some *Lilium* species, and eight days in some *Fritillaria* species (Bennett, 1972). Plants with large GS tend to be geophytes that store nutrients in an underground storage organ and undergo rapid seasonal growth through cell expansion in the early spring before returning to dormancy (Grime & Mowforth, 1982; Veselý *et al.*, 2012). Plants with large GS are also thought to be less common in extreme environments, whereas taxa with small GSs are distributed across a wider range of environmental conditions (Knight & Ackerly, 2002; Knight *et al.*, 2005). In addition, invasive and naturalised species tend to have smaller GS relative to non-invasive taxa (Kubešová *et al.*, 2010; Suda *et al.*, 2015) (although this is not always the case (Abbott & Forbes, 2002)). Species with a large GS are associated with an increased risk of extinction, where species of global concern are shown to have significantly larger GS than species of local and no concern (log back-transformed GS of approximately 5pg, 2.7pg, and 2.3 pg respectively), in particular taxa with lower ploidy

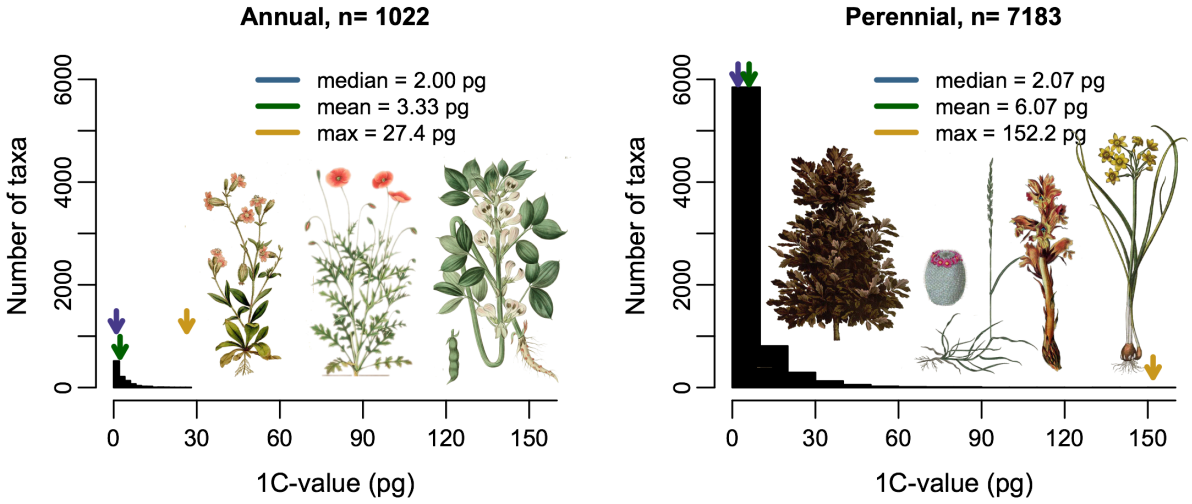

Figure 1. 3 Comparison of the range in genomes sizes between annual angiosperms and perennial angiosperms. Annual species shown, from left to right, are *Silene pendula* (Caryophyllaceae), 1C = 1.18; *Papaver dubium* (Papaveraceae), 1C = 4.5 pg; and *Vicia faba* (Fabaceae), 1C = 27.4 pg. Perennial plants from left to right are *Quercus robur* (Fagaceae), 1C = 0.9; *Mammillaria haageana*(Cactaceae), 1C = 1.56; *Lolium perenne* (Poaceae) 1C = 2.76; *Orobanche caryophyllacea* (Orobanchaceae), 1C = 3.56; and *Narcissus jonquilla* (Amaryllidaceae), 1C = 16.4 pg. C-values were obtained from the Plant DNA C-values database (Bennett & Leitch, 2012).

levels (Vinogradov, 2003). Also reported is a negative correlation between the number of species in a genus and average genome size of the genus (Knight *et al.*, 2005). This may be due to the influence of GS on cell division time, growth rate, and generation time, thus imposing ecological constraints (e.g. length of growing season) and greater extinction risk (Knight *et al.*, 2005).

Genomic plasticity

Angiosperms are distinctive in having unusually high levels of genomic plasticity, involving, for example, multiple rounds of whole genome duplications or polyploidy (i.e. more than two sets of chromosomes) in their ancestry and a high turnover of non-coding repetitive DNA (Leitch & Leitch, 2008), processes that can result in substantial GS diversity with species radiations (Kraaijeveld, 2010; Puttick *et al.*, 2015).

Polyploidy can arise by fertilization involving unreduced gametes. Nearly one-third (31.1%) of taxa with known ploidy levels and C-values are recognised as polyploids (n= 6339) (Bennett & Leitch, 2012), with levels as high as 38-ploid in the monocot *Poa litorosa* (Poaceae) (Murray *et al.*, 2005) and 80-ploid in the eudicot *Sedum suaveolens* (Crassulaceae). Polyploidy increases the amount of DNA in the genome, at least initially, which if GS does provide a selective pressure, should result in polyploid individuals being selected against. Yet, it is estimated that 15% of angiosperm speciation events are associated with polyploidy (Wood *et al.*, 2009). Furthermore, mapping paleopolyploidy events to phylogenetic trees has revealed that polyploidy occurred at the base of both seed plant and angiosperms radiations (Jiao *et al.*, 2011) and is pervasive throughout angiosperm evolutionary history, with at least 50 paleopolyploidy events known (Van de Peer *et al.*, 2009; Jiao *et al.*, 2011; Fawcett *et al.*, 2013).

Adaptive and fitness advantages could be benefits of polyploidy, perhaps through selective gene retention, fixed heterozygosity and enhanced allelic variation that makes them more competitive than diploid taxa. In a translocation experiment of *Achillea borealis* (Asteraceae), both established hexaploids and first-generation hexaploids had a higher survivorship than over tetraploid plants (five-fold and 70% respectively) (Ramsey, 2011). Polyploid taxa potentially have double the machinery to undertake fundamental cell processes with the same, or better efficiency as diploids (e.g. the two annual polyploids in Fig. 1.2 have shorter meiosis duration). Higher ploidy levels may confer additional advantages, as is reported with increased photosynthesis in octoploid C4 grasses relative to tetraploids, where the octoploids showed up to 70% higher enzyme activity (Warner *et al.*, 1987), perhaps owing to higher expression of genes in the Calvin cycle (Ilut *et al.*, 2012). Similarly, tetra-, penta- and hexaploids in geophytes (*Allium*) showed higher maximum photosynthesis rates with higher ploidy level (Ježilová *et al.*, 2015), though these were more subtle. Ecologically, polyploidy is associated with greater ability to tolerate nutrient stress, possibly due to superior ion

uptake efficiency; greater tolerance for drought due to increased capacity for retaining water; and greater resistance to cold and frost (Levin, 1983), with high incidences of polyploidy in alpine and arctic habitats (Brochmann *et al.*, 2004), for example *Poa litorosa* mentioned above, which is a sub-antarctic species.

Whilst polyploidy may confer adaptive advantages in some circumstances, it also gives rise to step increases in GS. However, an analysis of mean GSs of species at different ploidal levels, reveal that polyploids often lose DNA over time, a process called "genome downsizing" (Leitch & Bennett, 1997, 2004). Polyploidization is not the only process that increases GS. Transposable elements (TE) increase episodically and their differential proliferations are positively correlated with GS, with the percentage of the genome occupied by TEs ranging from 10% in *Medicago truncatula* (0.47 pg, Fabaceae) to 80% in wheat (17.3 pg, *Triticum aestivum*, Poaceae) and barley (5.5 pg, *Hordeum vulgare*, Poaceae) (reviewed in Grover & Wendel, 2010; Kejnovsky *et al.*, 2012). Indeed, TEs can proliferate very rapidly over short evolutionary time scales (Hawkins et al. 2008) and result in substantial genetic changes, which may lead to genetic isolation (Oliver *et al.*, 2013).

Very large genomes, such as those seen in *Fritillaria* (1C-values range from c. 30 – 100 pg), are marked by a large number of heterogeneous repeats, a low turnover of DNA and infrequent deletions (Kelly *et al.*, 2015). These taxa are geophytes, where selection for small genomes may be more relaxed. At the genomic level, an arms race can be expected between mechanisms of DNA loss, which may help to alleviate the costs of a large GS and competing forces for genome expansion, such as TE expansion.

Costly genomes

The minimum amount of DNA necessary to accommodate genes and indispensable regulatory sequences is estimated to be around 50 Mb for a plant (Bennett and Leitch 2005). Larger genomes are costly in terms of the amounts of nitrogen (N) and phosphates (P) needed to synthesise nucleic acids (Leitch & Bennett, 2004). Inorganic P

and N form the backbone of nucleic acids, comprised of 8.7% P, 14.5% N (Sterner & Elser, 2002). The synthesis of DNA must compete for P with the P demands of transcription, translation, photosynthesis, cellular metabolism, enzymatic activity and biochemical energy (ATP) (Westheimer, 1987), and the N demands of amino acids, proteins (17%) and nucleic acids (14.5%) (Novoa & Loomis, 1981; Sterner & Elser, 2002). Fast growth rates are particularly demanding, firstly for P to build ribosomes, and secondly for N to build proteins, and are associated with high levels of RNA. Ribosomes themselves are potentially a very significant repository of P in ecosystems (Elser *et al.*, 2003).

Despite the high cellular demands for N and P, these two macronutrients are often available in limited quantities in the ecosystem. Phosphate composes only 0.09 wt% of the earth's crust; it is released during rock weathering at rates of 0.05 to 1.0 kg/ha and concentrations in soil solution range from very high at 10^{-4} M to very low in some tropical regions at 10^{-8} M (Newman, 1995; Filippelli, 2008; Johnston *et al.*, 2014). The latitudinal differences in soil P concentrations (Reich & Oleksyn, 2004) may be driving patterns of broad geographical associations in herbaceous angiosperms between smaller GS in tropical regions, characterised by lower soil P and larger GS in temperate regions which typically have higher soil P concentrations (Levin & Funderburg, 1979). To make matters more difficult still for plants to assimilate P, is the observation that up to 80% of soil phosphate is in organic forms and must first be mineralised before it is available for uptake by the plant (Raghothama, 1999). Similarly, N must first be oxidized or reduced before it can be used (Novoa & Loomis, 1981), and plants must then further compete for these key elements with microorganisms, especially under conditions of nutrient limitation (Kaye & Hart, 1997).

Stoichiometry, the measurement of relative proportions of energy and key elements in organisms, can provide an understanding of the trade-offs that come with high growth rates and an organism's carbon : N : P ratio under different nutrient

availability. The growth rate hypothesis, which was built on observations that fast-growing zooplankton had higher percentages of P by dry weight than slower-growing zooplankton, states that the differential allocations of P to RNA under different growth regimes is a driving force behind the contrasting elemental ratios of organisms, in particular variations in P (Sterner & Elser, 2002). This concept was expanded by Hessen *et. al* (2010), who suggested that it can be expected that larger genomes will have higher P demands for DNA, which, under limited P availability, could restrict the allocation of P for RNA and slow down the growth rate. A reallocation of P from DNA to RNA could promote faster growth rates and hence provide a selective pressure towards more streamlined genomes (Hessen *et al.*, 2010). In plants, ecological support for these genome downsizing and genome streamlining hypotheses come from two investigations of plant abundances on two long-term natural experiments (Šmarda *et al.*, 2013; Guignard *et al.*, 2016), the latter being a key finding of this thesis. These studies report that the plants growing on the experimental plots where both N and P were added not only produced the most biomass but also had a higher mean GS than plants growing on low nutrient plots.

Aims and scope of the thesis

Very little is known about how GS influences the abundance and distribution of plants under nutrient limitation. The aim of this thesis is to investigate the hypothesis that there are ecological costs associated with possessing large genomes, due to the higher demands for N and P required to construct nucleic acids (as discussed above) and that this, in turn, impacts on (i) the ecology of the plant, influencing where and how it grows, (ii) the plant community composition, and (iii) consequently ecosytem dynamics. In the first step towards testing this hypothesis, I examine the above-ground biomass of plants, their GS and ploidy level under various treatments of nitrogen and phosphate fertilizer at the Park Grass Experiment at Rothamsted Institute. I incorporated plant competition as a factor by attributing Grime's three plant strategies to each species (Chapter 2). The theme of

GS, polyploidy, competition, and nutrient limitation effects and interactions on plant abundances is extended in Chapter 3 to test whether, and how, herbivory influences plant biomass on another long-term experiment, Nash's field at Silwood Park. Chapter 4 focuses on the associations between GS, ploidy level, and the resource requirements for nutrients, light and water (i.e. Ellenberg's indicator values). The final chapter (Chapter 5) discusses the implications of the results obtained in the thesis, and avenues for future research.

Chapter 2 Genome size and ploidy influence angiosperm species biomass under nitrogen and phosphorus limitation

Publication information

This chapter is published in New Phytologist, for which I was the lead author. Co-authors Richard Nichols and Rob Knell contributed statistical expertise. Catalina-Andreea Romila conducted the stomata size measurements. All authors revised, read, and approved the final manuscript.

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Summary

Angiosperm genome sizes (GS) range c. 2,400-fold, and since nucleic acids are amongst the most phosphorus (P) and nitrogen (N) demanding cellular biomolecules, we test the hypothesis that a key influence on plant biomass and species composition is the interaction between N and P availability and plant GS. We analysed the impact of different nutrient regimes on above-ground biomass of angiosperm species with different GS, ploidy level, and Grime's C-S-R plant strategies growing at the Park Grass Experiment (Rothamsted, UK), established in 1856. The biomass-weighted mean GS of species growing on plots with the addition of both N and P fertilizer were significantly higher than in plants growing on control plots and plots with either N or P. The plants on these N+P plots are dominated by polyploids with large GS and a competitive plant strategy. The results are consistent with our hypothesis that large genomes are costly to build and maintain under N and P limitation. Hence GS and ploidy are significant traits affecting biomass growth under different nutrient regimes, influencing plant community composition and ecosystem dynamics. We propose that GS is a critical factor needed in models that bridge the knowledge gap between biodiversity and ecosystem functioning.

Introduction

There is a huge diversity of genome sizes (GS) encountered in plants and a potential for this to influence a plant's nutrient demands, and hence its ability to grow in different environments differing in nutrient availability. Angiosperm GS (often referred to as 1C-values = the amount of DNA in the unreplicated gametic nucleus) range an astonishing c. 2,400-fold, from 1C = 0.06 picograms (pg) in *Genlisia tuberosa* (Fleischmann *et al.*, 2014) to 1C = 152.2 pg in *Paris japonica* (Pellicer *et al.*, 2010) (1 pg = 978 Mbp; the distribution of GS across > 7,000 angiosperm species is shown in Fig. S2.1*a*). Angiosperm genomic diversity is also marked by the prevalence of polyploidy in the ancestry of most, perhaps all lineages. In addition, 15% of speciation events are estimated to involve polyploidy (Wood *et al.*, 2009), and estimates based on chromosome counts range from 24% (Barker *et al.*, 2016) to 35% (Wood *et al.*, 2009; Barker *et al.*, 2016) of polyploid species within a genus, where baseline polyploid number of chromosomes was established as being 3.5 times the lowest haploid count of the genus.

Whilst much has been written on the role of nutrients on plant distribution (Aerts & Chapin, 2000; Craine *et al.*, 2002; Harpole *et al.*, 2011), the impact of GS has received little attention. In this paper we ask if species with large GS and/or high ploidy level are limited in their productivity by nitrogen (N) and phosphorus (P) nutrient availability. A potential underlying mechanism in limiting angiosperm biomass productivity in terms of GS is that nucleic acids require N and P and larger genomes may be more costly. Phosphorus occurs in plants as inorganic orthophosphate or as organic phosphate esters, of which 40-60% are nucleic acids (Veneklaas *et al.*, 2012) and N is another important component of DNA, which has a C:N:P ratio of 9.5 : 3.7 : 1 (Sterner & Elser, 2002). Furthermore, N and P are scarce in many unfertilized soils. Phosphorus concentrations in soil range from high (100 μM) to low (1 μM) or very low (0.01 μM), as found in some tropical soils (Johnston *et al.*, 2014). In contrast, phosphate concentrations in plant tissues are estimated to range between 5-20 mM, several orders of magnitude higher

than soil concentrations (Raghothama, 1999). Up to 80% of soil P is in organic form and must first be mineralised before it is available to plants for uptake, principally in the form of orthophosphate ions ($H_2PO_4^-$) (Raghothama, 1999). Similarly, N is generally found at concentrations of 1 mM to 0.1 mM; and, as for P, it must first be oxidized or reduced before it is accessible to plants (Novoa & Loomis, 1981). Inorganic phosphate has a low diffusion coefficient in soil and the production of an extensive root system for P scavenging, mining by secretion of carboxylates to solubilise inorganic P, or symbioses with mycorrhizal species are of particular importance in P acquisition (Lambers *et al.*, 2008; Richardson *et al.*, 2009). In contrast, N is more mobile and its uptake, either as NO_3^- or NH_4^+ occurs primarily via a combination of mass flow and diffusion (Richardson *et al.*, 2009).

To investigate the impact of N and P on the productivity of species as a function of GS and ploidy, we take advantage of the Park Grass Experiment, Rothamsted, UK, which is the world's longest continuously running ecological experiment, established in 1856 (see Materials and Methods). Previously it was shown from this site that fertilizer treatments significantly affected species composition and biomass (Crawley *et al.*, 2005). Here we build on that analysis to test the hypothesis that the biomass response to N and P fertilization is dependent on polyploidy level and GS. Specifically, we examine whether N and P availability, and their interaction, differentially affects a species ability to produce biomass dependent on (i) GS and (ii) polyploidy within the competitive setting of a grassland community.

Plant biodiversity and biomass are influenced by both abiotic (e.g. nutrient availability, shade, climate, atmospheric gases) and biotic factors (e.g. soil microorganism communities, competition, predation, pathogens). From the complexity of interactions, Grime (1977) identified three primary plant strategies (the C-S-R plant strategies) that provide both a synthesis of plant responses to environmental stress and predictive power of species occurrence at the community scale. These strategies are defined by the degree

to which a species has a propensity to be a competitor (C) for space and resources, is tolerant to environmental stress (S) or is a ruderal (R), the latter being a species which tends to be short-lived, weedy and tolerant to disturbance. To investigate the impact of N and P availability on plant biodiversity and biomass, this paper applies Grime's framework to address how ploidy level and GS are associated with C-S-R strategies (Grime, 1977) and how these in turn are influenced by N and P availability.

This work directly complements and extends the only other similar study (Šmarda *et al.*, 2013), which investigated the effect of P on GS and ploidy of plants growing at the Rengen grassland experiment in Germany, established in 1941. Although Šmarda *et al.*'s study demonstrated that P did indeed influence biomass production, due to the experimental design it was unable to estimate the effects of N and P separately. Here we demonstrate that N and P given separately have no effect on GS and ploidy distributions, but that it is their combined presence that is significant. We also demonstrate that the species that adopt a C-strategy at Park Grass are dominated by polyploids with large GS.

Recent work scaling information from local grassland surveys to provide insights into plant distributions over continental scales (Violle *et al.*, 2015) have highlighted the need to link our understanding of biodiversity with understanding of ecosystem function and resilience. This is especially important in the face of anthropogenic-induced stressors such as N and P eutrophication. We propose that GS and ploidy levels may represent important components of biodiversity that can be used to inform such models.

Materials and Methods

Study site

The Park Grass Experiment was established in 1856 on 2.8 ha of parkland at Rothamsted in South East England, UK, and is the longest continuously running ecological experiment in existence (Lawes *et al.*, 1882; Silvertown *et al.*, 2006). A detailed overview

of the history of fertilizer inputs to the site is given in Crawley *et al.* (2005) and nutrient regimes are summarized in Fig. S2.2. Briefly, for at least 100 years before its establishment, the experimental site was natural, unploughed grassland and fertilised occasionally with farmyard manure, road scrapings or guano. One crop of hay was removed annually and a second crop was eaten off by sheep (Lawes & Gilbert, 1859). However, the soil was of poor nutrient status and mildly acidic (pH 5.4-5.6) when the experiment started. The experiment comprises 20 main plots, each receiving specific combinations of mineral fertiliser or organic manures. In 1965 most of the plots were divided into four subplots (a, b, c & d), with three (a, b & c) receiving lime ($CaCO_3$), every three years, if necessary, to maintain soil pH levels at 7, 6, and 5. The fourth subplot (d) is unlimed and soil pH here varies from pH 3.6 to pH 5.7 depending on N fertilizer treatment. For consistency, we included only subplots with uninterrupted mineral fertilizer treatments for > 100 years and a pH > 4.5 in the analyses. In total, we analysed 64 subplots (16 plots and 15 treatment types), including 3 control plots. Two control plots were established in 1856 to 1863 and a third, which received farmyard manure from 1856 to 1863 can now be regarded as a control. The herbage on each plot is cut annually in mid to late June and again in autumn. On plots with fertilizer treatment, different combinations of N, P, potassium (K), sodium (Na), magnesium (Mg), and silica (Si) are applied. In terms of N and P nutrients, 11 subplots are treated with N but without P (= N subplots), 16 subplots are treated with P but without N (= P subplots), 25 subplots get both N and P treatment (= N+P subplots), and 12 subplots are control plots (i.e. receive no nutrient treatments). Nitrogen is applied either as $(NH_4)_2SO_4$, or as $NaNO_3$, in spring. The NH_4 treatment is applied at four different dosages: nil, low, mid, and high; the NO_3 is applied at 3 different dosages: nil, low, and mid. These dosages correspond to 0, 48, 96 and 144 kg of N applied per hectare, per year, respectively. Potassium is applied in combination with N on 4 (out of 11) subplots, 8 of the 16 subplots with P, and 19 of the 25 subplots with N+P. Silica is applied on 3 subplots with N+P. See Fig. S2.2 for a more detailed description. Phosphate, K, Na and Mg are applied in late autumn or winter.

Species at Park Grass

Crawley *et al.* (2005) identified sixty-one species on the 64 subplots and these are listed in Table S2.1 and S2.2. All but one (*Ophioglossum vulgatum*, Monilophyta) are angiosperms. Of the angiosperms, 21 are monocots (4 families) and 39 eudicots (14 families). *Ophioglossum vulgatum* occurred on just two subplots with a dry weight comprising less than one percent of subplot herbage yield and was removed from the analyses in order to focus on angiosperms.

All but four species studied at Park Grass are perennial (*Bromus hordeaceus,* annual-biennial; *Crepis capillaris*, annual-perennial; *Heracleum sphondylium*, biennial-perennial; *Tragopogon pratensis*, biennial).

Biomass, genome size and ploidy estimates

Species dry weights were taken from Crawley *et al.* (2005) and comprise data obtained from the above-ground herbage harvested from six small quadrats (50 x 25 cm) within each subplot, before the first cut of the season, from 1991 to 2000. The samples were then sorted into species, oven-dried and dry weights of each species obtained. The 10-year mean herbage yields represent the biomass estimates of each species used in this paper.

For GS and ploidy level estimations we collected fresh leaves in April-September 2013. We screened fourteen taxa (AM, AO, BH, BM, DG, FP, FR, HI, KA, LO, LP, PP, RA, TO; see Table S2.1 for abbreviations of species names) known to have two or more cytotypes (ploidy level) in Europe. Leaves from at least 12 plants of these species were collected where possible from control plots and from subplots with N+P, and/or midpoint N and P treatment, to examine whether different nutrient regimes selected for different cytotypes. Four taxa (*Ajuga reptans*, *Agrimonia eupatorium*, *Conopodium majus*, *Ononis repens*) without published GS estimates were sampled from plots where they were most common to determine their GS. We also screened three taxa (*Agrostis capillaris*, *Arrhenatherum*

elatius, *Holcus lanatus*) for cytotype variation, as these occurred in over 50% of the subplots and across low to high nutrient regimes. Where possible, leaves of the remaining taxa (i.e. taxa with published GS and a single known ploidy level) were sampled in October prior to the second cut to ensure we were using an appropriate C-value. GS estimates of taxa which we did not collect were obtained from the Plant DNA C-values database (Bennett & Leitch, 2012) (Table S2.2).

1C-values were estimated by flow cytometry using a Partec CyFlow Space fitted with a Cobalt Samba green (532 nm, 100 mW) laser. Approximately 20 mg of leaf or stem sample was co-chopped with either *Petroselinum crispum* "Champion Moss Curled" (1C=2.22 pg) or *Pisum sativum* "Minerva Maple" (1C=4.86 pg) as the internal calibration standard in General Purpose Buffer, LB01, Galbraith, Otto or Partec CyStain Absolute P buffer (depending on the species) as described in Pellicer & Leitch (2014). To screen for different ploidy levels within a species, tissue from up to six individual plants were co-chopped with an internal standard and run on the flow cytometer to measure 1000 or more nuclei. To report GS for AR, AU, CM, OR, tissue samples from three individual plants were analysed with each sample run three times, measuring the GS of 5000 or more nuclei per run. The mean coefficient of variation (CoV) of sample and standard peaks in the flow histograms are reported in Table S2.2. Ploidy levels were established as those that matched GS in the C-values database. If GS and/or ploidy level data were unavailable for a particular taxa, we looked at published GS and/or chromosome counts (Goldblatt P, 2012) for the taxa and species within the same genus.

Phylogeny

Evolutionary relationships between species were estimated to account for phylogenetic non-independence in the statistical analyses. A phylogenetic tree of species found at Park Grass was reconstructed with nucleotide sequences for the plastid markers atpF-atpH,

matK, rbcL, rps16, trnF-trnL and trnL-trnT obtained from Genbank (Benson *et al.*, 2013) (Table S2.1), aligned in MEGA 5.0 (Tamura *et al.*, 2011) using Muscle (Edgar, 2004), checked visually, and concatenated in SeaView (Gouy *et al.*, 2010). A maximum-likelihood (ML) phylogenetic tree was estimated (Fig. S2.3) and the position of one family (Caryophyllaceae) edited in MEGA to be consistent with the APG III angiosperm phylogeny (The Angiosperm Phylogeny Group 2009).

Statistical analyses

All analyses were carried out in R (R Development Core Team, 2012).

We first estimated the biomass-weighted mean 1C-value for each of the 64 subplots. This was achieved by summing the product of each species 1C-value with its biomass fraction (species subplot biomass/total subplot biomass) (Table 2.1a). For the boxplot displays (Fig. 2.1), we grouped the subplot biomass-weighted mean 1C-values into one of four categories (control, N, P, and N+P) dependent on the nutrient treatment. To test the effects of N and P on the biomass-weighted mean 1C-values of each subplot in a two-way analysis of variance (ANOVA) (Pinheiro *et al.*, 2013), we fitted a linear mixed effect (LME) model with the experimental N and P treatments scored as a 2x2 factorial N-/N+, P-/P+ , with subplot treated as a random effect.

We also fitted LME models to investigate the effect of each nutrient applied (i.e. N (NO_3 and NH_4), P, K, Si, Na) on biomass-weighted mean 1C-value, with subplot treated as random effect. The significance of each nutrient was tested by model reduction with ML inference and the final most parsimonious model was inferred with restricted ML (REML).

To examine the impact of ploidy and GS on plant biomass under nutrient limitation at the community level (i.e. individual subplots), we compared total subplot biomass and species numbers of: (i) species with large GS (1C ≥ 5 pg) vs species with small GS (1C < 5 pg) and; (ii) diploids vs polyploids, between the four levels of treatment: control, N, P, and N+P (scored as +N/-N, +P/-P). We also present results in the Supplementary

Information where we set the large GS threshold at ≥2.5 pg (i.e. the median GS for angiosperms, Leitch & Leitch (2013), including for those taxa at Park Grass (2.53 pg, Fig. S2.1)), ≥3 pg and ≥6 pg. We also consider GS as a continuous variable (see below). For all thresholds, species were partitioned into four genomic groups: 1) diploid taxa with small GS; 2) diploid taxa with large GS; 3) polyploid taxa with small GS; and 4) polyploid taxa with large GS. We tested to see whether the total biomass of each genomic group was dependent on different N and P treatment in an ANOVA model with interaction terms between GS, ploidy, N and P (subplot was treated as a random factor). Assignment of ploidy level was based on our GS estimations obtained in the present study and chromosome counts published in conjunction with C-value (Šmarda *et al.*, 2007; Bennett & Leitch, 2012; Rice *et al.*, 2015). We proceeded to compare biomass-weighted C-S-R strategies between these genomic groups. Biomass and species numbers were square-rooted to meet model assumptions of normality. C-S-R types were attributed to each taxon following Hodgson *et al.* (1999) (Table S2.2). Each species has a C: S: R ratio that sums to one, the numbers in the ratio being used to partition biomass data. For example, a species with a C-S-R category of 0.5: 0.25: 0.25 and 10 g of biomass was partitioned 5 g of biomass to "C" and 2.5 g weight to each of "S" and "R". To test the significance of GS, ploidy, and fertilizer on differential biomass among the C-S-R strategies, we performed a multivariate ANOVA with interaction terms between all main effects (i.e. GS, ploidy, N, and P, all as binary factors). Because a combination of C-S-R strategies was attributed to each species, we did not analyse species numbers for each strategy.

We tested for evidence of phylogenetic signal in the GS data and the C-S-R strategies (i.e. non-independence between phylogeny and e.g. GS) with the function *phylosig* (Revell, 2012). Phylogenetic signal is tested for by comparing the distribution of the trait in question against randomised phylogenetic relationships. No phylogenetic signal was detected in the C-S-R data, but a significant phylogenetic signal was present among species for GS (K-stat = 2.835, P=0.001, lambda = 1.049). To account for phylogenetic non-independence, we fitted phylogenetic generalised linear mixed models (PGLMM)

with Markov chain Monte Carlo techniques (MCMCglmm) (Hadfield, 2010). We tested the effects of GS, ploidy level, N and P treatments and how interactions between these variables contributed to species biomass across all subplots, whilst allowing for phylogenetic correlations. Ploidy, N, and P were treated as binary variables. Biomass and 1C-values were $\log_{10}$ transformed to ensure normality of errors. We treated subplot and species identity as random effects, and phylogeny as a covariance structure (see Methods S1 for phylogenetic tree file). Models were run with five million iterations including a burn-in of 8,000 and a thinning interval of 500, resulting in effective sampling sizes from 9370 to 9984 for all variables and interactions, including random variables. We tested the effect of different priors (e.g. flat (nu=0), weak (e.g. nu=0.002, V=1), and expanded priors (alpha.mu=0, alpha.V=1000) and found that these had no, or only very small effects on the estimated means and significance of the parameters. We report here the parameter estimates with a prior where *nu* = 0.5 and each variance component = 1 as this had the best convergence and chain mixing.

In line with Šmarda *et al.* (2013), we also estimated biomass-weighted mean 1C-values using the phylogenetic generalized least squares (PGLS) method (Paradis *et al.*, 2004, Pinheiro *et al.*, 2013), which we include in Supplementary Information (Fig. S2.4 and Table S2.3).

Results

GS and ploidy diversity of species growing at Park Grass

Genome size ranges 157-fold amongst the 60 angiosperm species on the plots we analysed, from 0.3 pg in *Carex flacca* to 47.3 pg in *Fritillaria meleagris*; with a median and mean of 2.53 and 4.07 pg respectively (Fig. S2.1*b*). The only taxon we found with a range of GS was *Poa pratensis* (1C-value = 3.3 to 7 pg). This species is known to have an extensive variation in chromosome numbers (Rice *et al.*, 2015). We used a mean GS of 1C = 4.9 pg for this species (Table S2.2). We report a new cytotype 1C-value for *Lathyrus*

pratensis at 11.46 pg, the previous reported range being 4.54 -7.35 pg (Bennett & Leitch, 2012).

Subplot biomass-weighted mean genome size

To investigate whether GS contributes to the differential biomass across subplots, we first determined the biomass-weighted mean 1C-values for each of four nutrient treatments (control, N, P, N+P) and indicate an increased mean with N+P (Fig. 2.1, Table 2.1a). Two-way ANOVAs showed that the subplot biomass-weighted mean 1C-value increased only under the addition of both N and P (F (1, 60) =26.82, p<0.0001, Table 2.1b). Treatment contrasts showed that the biomass-weighted mean 1C-values increased from 3.99 pg on control plots to 5.4 pg on plots with both N and P treatment (Table 2.1b).

To determine whether these results were primarily due to the addition of N+P or whether other nutrients added were also having an effect and influencing the results, we used linear mixed effect (LME) models with subplot as a random effect. We found that P and

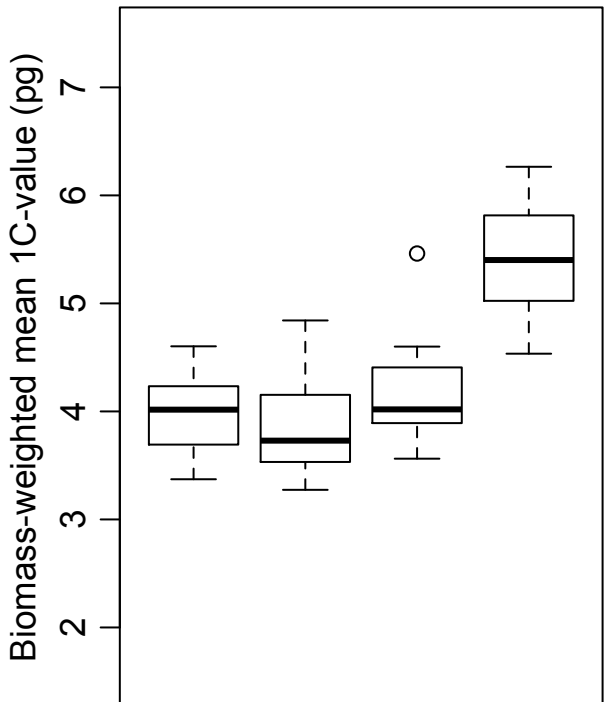

Figure 2. 1 Boxplot showing biomass-weighted mean 1C-values of subplots under each of the four fertilizer treatments: control (no N or P added), N (N without P), P (P without N), N+P (both N and P added), number of subplots per treatment: control, 12 plots; N, 11 plots; P, 16 plots; N+P, 25 plots. See also Tables 2.1a and S2.3 for measures of simple

NH_4, and NO_3 were highly significantly influencing biomass-weighted mean 1C-values (Table 2.1c). Silica also has a significant effect (p=0.0299), but it is only applied on one plot with high levels of N+P treatment. Given these results, we feel justified in combining the data from the different subplots into the four nutrient categories (i.e. control, N, P and N+P) used above.

Effects of GS, polyploidy, and nutrients on biomass and species numbers

The species biomass data were split into biomass from (i) species with small (1C< 5pg) and large (1C ≥5 pg) GS and (ii) diploid and polyploid species (Fig. 2.2). In terms of simple biomass ratios, plants with small GS comprised around two-thirds of the total biomass in the control, N, and P plots (0.65, 0.72, 0.66 respectively), whereas in plots

with N+P, plants with large GS contributed more than half (0.59) of the total biomass (Fig. 2.2a, Table S2.4a). Polyploid taxa dominated biomass under all treatments (0.7, 0.68, 0.61, 0.75 on control, N, P, N+P; Fig. 2.2c, Table S2.4). ANOVAs show that GS (F (1, 180) = 111.17), and ploidy (F (1, 180) = 361.88) both have highly significant effects (p<0.0001) on plant biomass, and that these two genomic parameters interact with N and P (F (1, 180) = 11.8, p= 0.0007). Treatment contrasts show this four-way interaction (N : P : GS : ploidy) has the largest effect on biomass (Table S2.5, S2.6, and see also below). This result is shown visually by splitting the data into four genomic groups (Fig. 2.2e). The graph shows that increased biomass is associated with polyploids of large GS on N+P plots (mean biomass ratio = 0.584, Table S2.4a). Across all nutrient treatments, diploid and polyploid species with small GS made similar contributions to biomass, while diploid species with large GS generated little biomass under any treatment (e.g. *Helictotrichon pubescens*, *Ranunculus bulbosus,* and *Fritillaria meleagris*). (See Table S2.4 for means, standard deviations, and ratios of total biomass and species numbers, Table S2.5 for ANOVA statistics; Fig. S2.5 for boxplots of Figs 2.2*a, c, e*; Fig. S2.6 for boxplots of Figs 2.2*b, d, f*). Three-way interactions between P, GS and ploidy level and

Table 2. 1 (**a**) Means and standard deviations of total biomass, species number, and biomass-weighted mean 1C-values of the subplots according to nutrient treatment. (**b**) Treatment contrasts and ANOVA statistical output testing the effect of N and P and their interaction (N : P) on subplot total biomass-weighted 1C-values of subplots. The estimated coefficients in the second column show the effects of N application (i.e. without P), the effects of P application (i.e. without N), and the effects when both are applied on a subplot, where the reference level is no application of N or P (i.e. control). (**c**) Linear mixed effect models significance (p-value) showing influence of nutrients on biomass-weighted mean 1C-value. Significant parameters are in bold. DF= degrees of freedom.

a)

Treatment	n	Total biomass (g)	Species number	Biomass-weighted mean 1C-value (pg)
Control	12	31.71±4.27	39±4.2	3.99±0.37
N	11	34.47±3.9	31±5.6	3.87±0.51
P	16	44.49±11.75	32±4.2	4.17±0.44
N+P	25	58.36±11.64	20±5.5	5.40±0.52

b)

	Estimate	Std. error	t value	Pr(>|t|)	DF	F-value	p-value
Intercept	3.984	0.14	28.374	<0.00001	1, 60	5639.792	<0.0001
N	-0.11	0.203	-0.543	0.589	**1, 60**	**47.589**	**<0.0001**
P	0.164	0.188	0.869	0.388	**1, 60**	**46.97**	**<0.0001**
N : P	**1.331**	**0.257**	**5.178**	**<0.00001**	**1, 60**	**26.816**	**<0.0001**

c)

Nutrient	Biomass-weighted mean 1C-value
Si	**0.0299**
Na & Mg	0.1367
K	0.9221
P	**<0.0001**
NO_3	**<0.0001**
NH_4	**<0.0001**

four-way interactions involving also N remain highly significant when different thresholds (1C ≥ 2.5, 3, and 6 pg) are used to delimit large GS (Figs S2.7-S2.9, Tables S2.7 – S2.12).

The total number of species on plots with N+P decreased as previously reported (Crawley et al. 2005). Species diversity in each of the four genomic groups differed significantly (i.e. diploids with large and small GS and polyploids with large or small GS). Treatment contrasts show GS (F (1, 180) = 1719.7, p <0.0001) and interactions between GS : ploidy ((F (1, 180) = 1227.5, p <0.0001) have the greatest influence on species diversity (threshold for large genome 1C ≥ 5pg; Fig. 2.2*b, d* and *f*, Tables S2.4-S2.5, Fig. S2.6). The same was true when using a large GS threshold of ≥2.5 pg and 6 pg. However, for a threshold of ≥3 pg, whilst the interaction between GS : ploidy also had the strongest effect on species diversity (p<0.0001) and GS a significant effect (p<0.0003), the second strongest influence was N (see Tables S.27-S2.12).

Testing the impact of C-S-R strategies on biomass production under different nutrient regimes

We investigated whether Grime's (1977) plant strategy categories (i.e. C-competitors, S-stress tolerators, and R-ruderals ("weeds")) contributed to the distribution of biomass on the Park Grass subplots. It is already known that the addition of fertilizers favours plants with a competitive strategy (Grime 1977). To determine the effect of these strategies on biomass at Park Grass, we used published C-S-R ratios (Hodgson *et al.*, 1999) to weight species' biomass on each subplot (as described above for weighting the GS data). We then replotted our data for plants in the four genomic categories (i.e. diploids with large and small GS and polyploids with large or small GS) against plant strategy-weighted biomass.

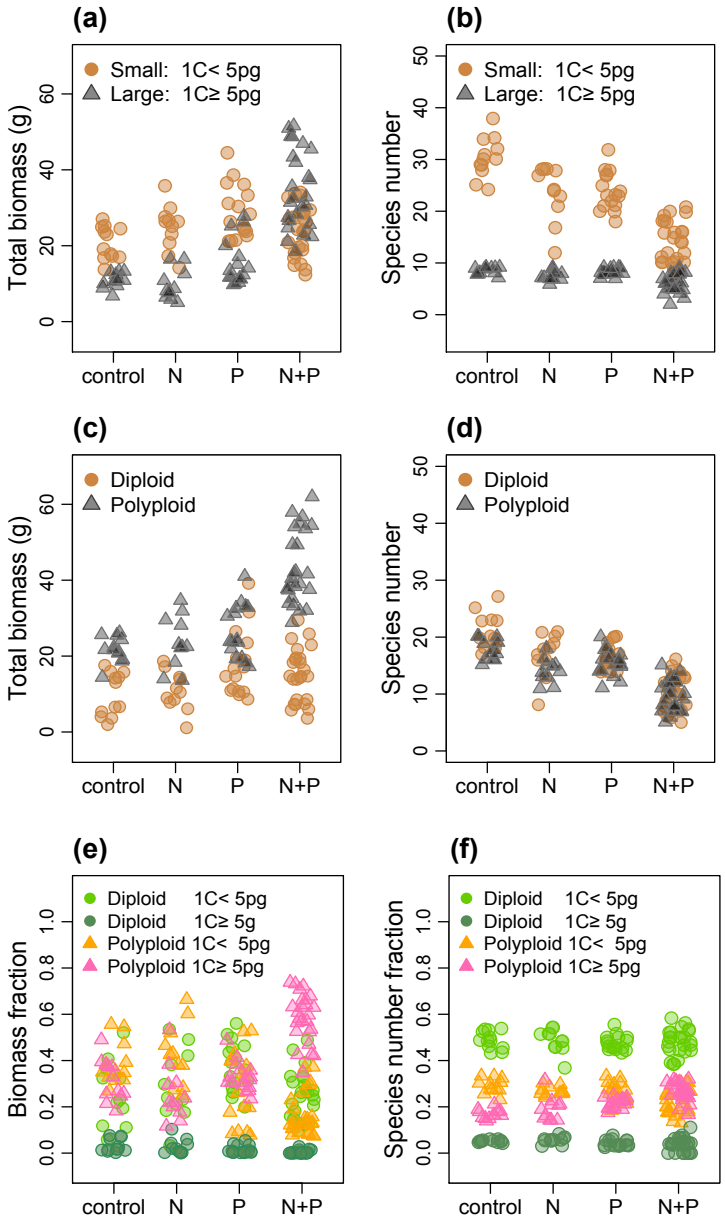

Figure 2. 2 Biomass and species numbers by genomic group. Graphs (a) and (b) show the impact of small GS (1C-value < 5 pg) vs large GS (1C ≥ 5 pg)); (c) and (d) show ploidy level (diploid vs polyploidy) on total biomass and total number of species respectively. Graphs (e) and (f) show the biomass and species number ratio of the four genomic groups: i) diploid taxa with small GS, ii) diploid taxa with large GS; iii) polyploids with small GS; and iv) polyploids with large GS. In a-d each subplot is represented by two points, and in (e), (f) by four points, one for each of the genomic groups. See Tables 2.1 and S2.3 and S2.5 for biomass and species number, ratios, means, standard deviations; also figures S2.5 and S2.6.

Most apparent was that N+P subplots were dominated by C-strategists which were polyploids with a large GS (see Fig. 2.3 and Table S2.5d – and there were significant interactions of N : P : GS : ploidy, F (1, 180) = 16.50, p<0.0001). These large-genomed, polyploid C-strategist species were

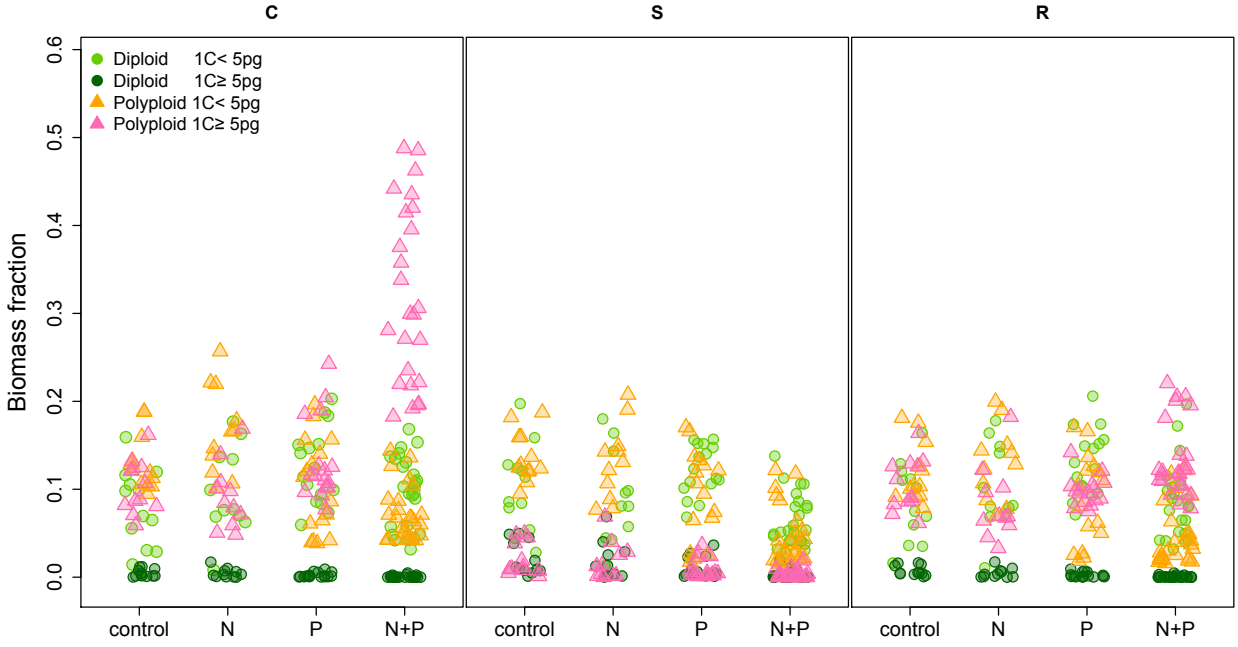

Figure 2. 3 The biomass data shown in Fig. 2.2 weighted by C-S-R strategy (C, competitor; S, stress-tolerant; R, ruderal). As in Fig. 2.2(e) and 2(f), each subplot is represented by four points corresponding to the four genomic parameter groups, with the exception of seven N+P subplots where diploids with 1C ≥ 5 pg were not present and thus are represented by only three data points. See also Table S2.4.

shown to comprise on average about one third of the total biomass of N+P plots (0.32±0.1, Fig. 2.3, Table S2.4c) and included two grasses *Arrhenatherum elatius* and *Alopecurus pratensis*, which contributed as much as 48.77% and 34.75% of biomass respectively. (Alternative thresholds to define large GS also revealed that the most productive C-strategists were polyploids with a large GS; Figs S2.10-S2.12, Tables S2.7–S2.12). For all thresholds of large GS (Tables S2.7–S2.12), the interaction between GS and ploidy had the largest positive effect on biomass productivity of S-taxa (e.g. 1C≥ 5 pg

F (1, 180) = 349.74, p< 0.0001) and R-taxa (e.g. 1C≥ 5 pg *F* (1, 180) = 504.96, p< 0.0001).

Testing the effects of GS, ploidy level, and N and P treatment using Bayesian approaches

Whilst the results above are consistent with our assertion that plant strategy, polyploidy and GS interact together to influence biomass and species composition, dependent on fertilizer input, we also analysed our data using a Bayesian PGLMM, to address the possibility that results are distorted by the non-independent response of phylogenetically related species amongst subplots. *MCMCglmm* confirms that increased biomass involves a significant (p = 0.028, Table 2.2) three-way interaction between GS, N and P. Four-way interactions involving GS, N, P and ploidy are not significant, but three-way interactions with GS, P and ploidy are significant (p <0.0001, Table 2.2). P and pH also have significant positive influences on biomass (Table 2.2). Interestingly, the two-way interactions between P : ploidy and P : GS are significantly negative, meaning that the addition of P without N, is associated with small GS diploids increasing their biomass productivity over large GS polyploids. Thus, collectively, these data indicate that biomass production varies not only with fertilizer treatment, but also with its interaction with GS and ploidy level.

For completeness and comparisons with Šmarda *et al.* (2013), as an alternative approach to factor in phylogenetic non-independence of the data, we also analysed the data using the phylogenetic generalized least squares (PGLS) method (Fig. S2.4, Tables S2.3, S2.6). The output was qualitatively the same as we observe through analyses of biomass-weighted mean 1C-value and *MCMCglmm* outlined above.

Table 2. 2 (**a**) Effects of genome size (1C-value), ploidy, N and P on biomass yield at the Park Grass experiment. Phylogenetic generalized linear mixed model (*MCMCglmm*) coefficients (posterior mean), lower and upper 95% credible intervals of parameters, the effective sample size taken from the chain, with significant p-values ($p \leq 0.05$ in bold). (**b**) Statistics are shown for the (co)variance matrices of the random effects (G-structure) and the (co)variance matrix of the residuals (R-structure).

a)

	Posterior mean	95% CI	Eff. sample size	pMCMC
Intercept	-4.573	-6.11, -3.00	9984	< 0.0001
GS	0.186	-0.55, 0.95	9984	0.6202
N	-0.034	-0.57, 0.51	9370	0.905
P	**0.907**	0.42, 1.39	**9984**	**< 0.0001**
Ploidy	0.452	-1.01, 1.79	9984	0.5234
pH	**0.256**	0.12, 0.4	**9984**	**0.0012**
GS : N	0.04	-0.42, 0.49	9984	0.862
GS : P	**-0.536**	-0.95, -0.12	**9984**	**0.013**
N : P	-0.605	-1.37, 0.08	9540	0.098
GS : Ploidy	0.053	-1.05, 1.06	9984	0.9173
N : Ploidy	-0.722	-1.52, 0.03	9984	0.0693
P : Ploidy	**-1.411**	-2.15, -0.69	**9984**	**< 0.0001**
GS : N : P	**0.71**	0.07, 1.34	**9984**	**0.028**
GS : N : Ploidy	0.503	-0.09, 1.14	9671	0.1078
GS : P : Ploidy	**1.225**	0.63, 1.78	**9984**	**< 0.0001**
N : P : Ploidy	-0.872	-2.08, 0.4	9984	0.1735
GS : N : P : Ploidy	0.043	-0.87, 0.96	9984	0.9247

b)

	Posterior mean	95% CI	Eff sample size
G-structure: ~subplot	0.102	0.04, 0.17	9984
G-structure: ~phylogeny	0.818	0.04, 2.54	9984
G-structure: ~species	2.806	1.62, 4.09	9984
R-structure: ~units	3.022	2.83, 3.23	9984

Discussion

Influence of GS on plant biomass under different nutrient inputs

We show that the biomass-weighted mean 1C-value and the ploidy level of species growing in the presence of N+P fertilizer are significantly higher than for species on subplots without both these macronutrients (Figs 2.1 and 2.2). We also show that there is no such response when N and P are added on their own, i.e. that the increased biomass from species with large GS and/or polyploidy requires both these nutrients together. The *MCMCglmm* analysis indicates that GS and ploidy are significant in predicting species biomass dependent on nutrient status.

Soil pH was also shown to influence biomass and this effect may arise through its known impact on nutrient availability. At neutral pH ammonium (NH_4) is more rapidly converted to nitrate (NO_3^-) by soil microbes, and N fixation by *Rhizobium* in legumes declines with soil acidity. In addition, phosphate forms stable, insoluble minerals and is most available at neutral to slightly acidic pH (Lucas & Davis, 1961; Jensen, 2010). Further, acid conditions can solubilise soil aluminium, which can be toxic to plants, and favour aluminium-tolerant plant species, including at Park Grass (Gould *et al.*, 2014).

As with Park Grass, a similar response to combined N+P was observed in the Rengen grassland experiment in Germany, established in 1941 (Šmarda *et al.*, 2013), although that experiment could not dissect the individual impact of N and P as the experimental design did not include plots where N and P were applied separately. Nevertheless, our second demonstration of the impact of GS and ploidy in influencing biomass growth under different nutrient regimes may point to a general ecological response to N and P availability in the environment. Both experiments have shown that when angiosperms are released from N and P limitation there is an associated increase in biomass of species with large genomes, a phenomenon associated with increased biomass generated by polyploid taxa (see below). These results agree with observations showing that the combined input of N and P into terrestrial, aquatic, and marine environments produces

much stronger responses in plant community biomass production than N and P alone (Elser *et al.*, 2007; Harpole *et al.*, 2011), although such studies did not investigate the impact of GS and ploidy levels.

The requirements of N and P are clearly interlinked even though the properties of these two elements differ. Cellular processes such as transcription and translation require a coupling of N and P, where P is needed for mRNA synthesis, followed by translation which requires N for protein production. An increase in N facilitates the production of phosphatase enzymes that cleave ester-P bonds in soil to increase rates of P uptake (Vitousek *et al.*, 2010; Marklein & Houlton, 2012), while the availability of P is known to influence the rates of N fixation or denitrification (Sterner & Elser, 2002). At the genomic level, transcription factors that suppress primary root growth may be regulated by both N and P (Medici *et al.*, 2015). Furthermore, while photosynthetic capacity has often been shown to be related to leaf N concentrations, such a relationship is constrained in P-limited environments, possibly due to limitations of ribulose-1,5-bisphosphate (RuP) or ribulose-1,5-bisphosphate carboxylase (RuBisCO) regeneration in plants which are P-deficient (Reich *et al.*, 2009). Taken together, such interactions may well contribute to explaining why it is only when both N and P are added together that a significant increase in biomass is observed.

However, it is also necessary to explain the significant impact of GS and ploidy in influencing the plant response to N and P. Currently, how the large 2,400-fold range in angiosperm GS influences N and P demands in the plant is unknown, yet GS is likely to have significant resource implications because nucleic acids are amongst the most N and P demanding biomolecules of the cell, being approximately 39% N and nearly 9% P by mass. DNA must also be packaged in the nucleus, which requires N-demanding histones, one of the most abundant proteins of the cell (Sterner & Elser, 2002). That N-demand may also lead to trade-offs for N with RuBisCO.

Given our observation that N and P impact species composition dependent on GS and ploidy, it suggests that DNA is demanding for these nutrients. However, it is also known that cell size (Hodgson et al. 2010) and other factors (e.g. growth rates, cell division time, see review in Greilhuber & Leitch, 2013) correlate with GS across the range of GS found in angiosperms. Indeed at Park Grass guard cell size correlates with GS (Fig. S2.13). Potentially, increased N and P demands associated with GS at a cellular level could be offset by a reduction in the total number of cells in a tissue or overall, leading to altered metabolism, growth rates or RNA abundance (Coate & Doyle 2015). As far as we are aware, no data on total plant N and P associated with C-value have yet been obtained. To calculate that total is non-trivial, since it requires knowledge of the N and P loading in all tissues (roots, stems, leaves), the biomass of these tissues, and will vary with macronutrient availability in the soil and ontogenetic stage of the plant. Such calculations would best be derived under limiting nutrient conditions, to offset against N and P storage systems. To add further complications, because GS correlates with cell size, there may also be additional effects on photosynthesis efficiency, because increased cell size alters the dynamics of gas exchange in the leaf (Drake et al., 2013). Collectively, increases in GS probably lead to trade-offs in resource allocation between cellular compartments, resulting in altered growth parameters, life strategies and ecology under different nutrient regimes.

Selection against polyploids in limited-nutrient conditions

Angiosperm evolution is associated with multiple rounds of polyploidy, indeed even apparently diploid species are now considered to be paleopolyploids (Van de Peer *et al.*, 2009; Jiao *et al.*, 2011; Renny-Byfield & Wendel, 2014). Allopolyploids (produced by interspecific hybridization, and genome doubling) may benefit from hybrid vigour and fixed heterozygosity (Chen, 2010), and the evolution of novel 'transgressive' characters (Rieseberg & Willis, 2007). Furthermore, the duplicated gene copies in polyploids are freed from selective constraints, potentially enabling the evolution of new functions

(Soltis & Soltis, 2000; Soltis *et al.*, 2009). Polyploids are often associated with broader ecological niches and/or invasiveness leading to greater evolutionary success than diploids (Hegarty & Hiscock, 2008; te Beest *et al.*, 2012). Certainly, this combination of advantageous characters may contribute to explaining why polyploid taxa with large GS dominate total biomass under high nutrient (N+P) conditions, especially in contrast to diploid taxa with large GS (Fig. 2.2*e*). Overall, the higher biomass of polyploids at Park Grass suggests a disparity in productivity between diploids and polyploids. However diploids and polyploids with a GS < 5 pg have similar biomass (Fig. 2.3) and the shift in biomass ratios of polyploid: diploid taxa from about 2:1 on low nutrient and control plots to about 3:1 on N+P plots suggests that ploidy level alone is not sufficient to determine what constitutes a highly successful polyploid. Instead, the observed distribution and abundance of different plant species at Park Grass are the result of more complex processes influenced by (i) effects of GS on growth and (ii) competitiveness of polyploids, both mediated through interactions with N and P. We observed also significant increase in biomass of diploid plants with a small genome associated with the application of P without N (Table 2.2), perhaps because these plants are less N demanding and can better utilise available P. Thus paramount amongst the costs of high GS polyploids may be the increased biochemical demand for cellular N and P generated by GS multiplication. These costs should be considered alongside the more widely acknowledged costs associated with polyploidy, such as minority cytotype exclusion (Otto, 2007) and chromosome pairing problems in meiosis (Comai, 2005), which can also lead to polyploids having lower fitness compared with diploid taxa (Burton & Husband, 2000).

These data are consistent with the hypothesis that polyploids with large GS are demanding of N and P. Potentially, the increased nuclear demands for N and P could be offset by altering the total volume of RNA in the transcriptome. For example, it is known that the "genomic shock" generated by *de novo* polyploidy results in plants with variable transcriptome volumes (Grover *et al.*, 2012). Selection under limiting N and/or P could favour RNA-efficient variants with smaller total transcriptome volumes and/or RNA

transcripts that are less N-demanding (Acquisti *et al.*, 2009a, b). Nevertheless, the increased biomass of polyploid species on subplots receiving N+P (especially those with large genomes) suggests that polyploids on other subplots at Park Grass are under nutrient limitation.

Competitor taxa are predominantly polyploids with large genomes

Plants are typically limited by multiple resources, including competition for nutrients, space and light and have evolved strategies to overcome these limitations. Of these, species adopting competitive strategies (C-taxa), as described by Grime's C-S-R strategies (Grime, 1977) were expected to dominate on high nutrient plots. Indeed, this is what we observed at Park Grass, but in addition we see that the C-taxa dominating the N+P subplots tend to be polyploids with large (1C ≥ 5pg) genomes (Fig. 2.3). Of these species, those that are also found on control or low-nutrient plots produce only limited biomass and show no competitive advantage (e.g. *Arrhenatherum elatius* and *Alopecurus pratensis*).

We suggest that there may be an upper threshold in GS for species with a competitive growth strategy, since we suspect that species with very large GS (1C >35 pg, as defined in Leitch *et al.*, 1998) are predominantly stress-tolerant (S-taxa), limited to a slow-growing, long-lived life history. This hypothesis needs to be formally tested. From our data, the only species at Park Grass with a very large GS was *Fritillaria meleagris*, a slow growing bulbous diploid with a 1C-value of 47.3 pg (more than four times larger than the next largest GS at Park Grass). While one might expect this species to thrive in subplots with N+P due to the high N and P demands for maintaining such a large genome, it was only found in subplots with just N, suggesting it is unable to compete with the fast-growing C-taxa when both N and P are present in the subplots. Instead this species is probably limited by factors other than nutrient availability, and perhaps this has led to drift in its GS to its current astonishingly large size. GS itself may now constrain the adaptive potential of *F. meleagris*, because of the effect of GS on cell division rates

(Bennett, 1972; Cavalier-Smith, 2005; Knight *et al.*, 2005; Greilhuber & Leitch, 2013; Veselý *et al.*, 2013).

Global scope and scaling to landscape levels

Our data show that plants respond differently to environmental availability of both N and P, dependent on GS and ploidy. If the results reported here, together with those of Šmarda et al. (2013) are generalities, then there are significant ecological implications to our understanding of plant assemblages, distributions and occurrences. Potentially the patterns we observe occur at multiple scales, up to, and including, continental scales, all of which are influenced by e.g. underlying geologies, soil types, soil age, soil pH, and farming practices, and all with their own N and P dynamics. Those dynamics will provide selective pressures on species and their evolution, shaping species communities. It has been shown that trait-based studies can be used in models aiming to link community structures with ecosystem functioning (Violle *et al.*, 2015). In addition it has been suggested that GS is a trait that could be incorporated into such models (Suda *et al.*, 2015). Because the Park Grass Experiment is the longest continuously running field trial, we are able to detect significant measurable impacts of GS and ploidy on community structure depending on nutrient availability. We recommend that the Plant DNA C-values database (http://data.kew.org/cvalues/) is integrated to the TRY Plant Trait database (https://www.try-db.org/TryWeb/Home.php) to facilitate future studies. We anticipate that this will improve the predictive power of models and will enable us to determine better the role that GS plays in community structure and ecosystem functioning.

Chapter 3 Linking genome size, macronutrient availability, and herbivore effects on plant biomass in an experimental grassland

Publication information

This chapter is formatted for submission, the journal yet undecided. Michael J. Crawley provided the plant biomass data. Dasha Kovalenko conducted the majority of the flow cytometry work. All authors will read, revise and approve the manuscript.

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Summary

Angiosperm genome size, which shows astounding variation, can have ecological consequences by influencing species abundance, competitiveness, and productivity. We hypothesis that GS may also play a role at the trophic level by influencing plant responses to herbivory, and/or herbivore choice. We examine interactions between GS, nitrogen, phosphate, competition, and herbivory (rabbits, insects, molluscs) at a grassland experiment established in 1992. Using phylogenetically informed models and path analyses, we show that plants on rabbit-grazed plots have lower GS, especially in the absence of N. In contrast, mean plant GS increases on mollusc-grazed plots with N fertilizer. Overall, we demonstrate that GS and ploidy level play a key role in linking environmental N and P with species productivity and plant community composition. We recommend that these parameters be incorporated into models seeking to understand the flow of nutrients through food webs and plant resilience in the face of environmental change.

Introduction

Plant traits influence and mediate plant responses to environmental conditions, which include stresses arising from resource limitation, competition from neighbouring plants, and predation (Eskelinen *et al.*, 2012; Koerner *et al.*, 2014). Since plants form the basis of terrestrial food chains, factors that influence plant abundance and productivity may have implications onto subsequent trophic levels. The genome size (GS) and ploidy level of plant taxa have been shown to influence plant abundance under different conditions of macronutrient limitation. Previous research on two independent long-term grassland experiments (Šmarda *et al.*, 2013; Guignard *et al.*, 2016) have revealed that plant community biomass production is dependent upon interactions between nitrogen (N) and phosphate (P) input, GS, ploidy level, and growth strategy (cf. Grime's (Grime, 1977) competitive, stress-tolerant, and ruderal (C-S-R) growth strategies). The authors argued that large genomes are more costly to build and maintain, in terms of N and P, than small genomes because these elements are essential for the production of nucleic acids, yet are of limited availability in most ecosystems (Acquisti *et al.*, 2009a; Hessen *et al.*, 2010). Trade-offs may therefore exist for the use of resources, particularly N and P, between the production of biomass, and maintaining a larger genome.

While ecological dynamics are known to be shaped by a complex network of interactions between many factors including, for example, elemental resources, primary producers, and consumers (Hunter & Price, 1992; Power, 1992; Scherber *et al.*, 2010), we are completely ignorant as to whether GS plays a role in shaping interactions between plants and herbivore taxa, which may also be limited by N and P (Mattson Jr, 1980; Elser *et al.*, 2000; Denno & Fagan, 2003). Herbivores can be selective feeders (Miller, 1968; Bruelheide & Scheidel, 1999; Nisi *et al.*, 2015; Averill *et al.*, 2016) and may alter their feeding strategies in terms of nutrient content (Miller 1968; Milchunas *et al.* 1988; Pérez-Harguindeguy *et al.* 2003). There are intriguing indications that a link between GS and herbivory does exist. For example, such a link may explain why some herbivorous insects

favour polyploid over diploid variants of the same species (Nuismer & Thompson, 2001; Thompson *et al.*, 2004; Münzbergová, 2006; Münzbergová *et al.*, 2015). Similar results have also been observed with cows grazing preferentially on tetraploid versus diploid cytotypes of *Lolium perenne* (Balocchi & López, 2009). Certainly herbivory action directly affects species richness, plant biomass, and microhabitat, and indirectly affects competition dynamics within plant communities (Crawley, 1983; Olff & Ritchie, 1998). Increased tolerance to herbivore damage involves increased photosynthesis, rapid growth rates, carbon and nutrient reallocation (Strauss & Agrawal, 1999), all of which may be more costly and less efficient in taxa with large GS, particularly in conditions where N and/or P are limiting. Nutrient availability determines not only plant productivity, but also influences the effects of herbivores on plant communities (Mattson Jr, 1980; Bryant *et al.*, 1983; Price, 1991; Throop & Lerdau, 2004; Laliberté & Tylianakis, 2012). Grazers can increase plant diversity on rich soils, and decrease it on poor soils (Olff & Ritchie, 1998). Higher soil N concentrations leads to decreased C:N ratios in the plant, thus increasing plant palatability, although many of these plants are fast growing taxa able to compensate for damage caused by herbivory. On rich soils, herbivores can thus increase diversity, by keeping fast-growing plants in check and promoting the growth of less competitive, but better defended taxa (Price, 1991; Olff & Ritchie, 1998).

Plant traits that have been shown to affect herbivore preferences include plant architecture (Carmona *et al.*, 2011; Barbour *et al.*, 2015), life history (Diaz *et al.* 2007; Carmona *et al.* 2011) the presence of secondary metabolites and phenolics (Agrawal & Weber, 2015; Barbour *et al.*, 2015), plant height (Laliberte *et al.*, 2012), and foliage carbon (C):N ratios (Karban & Myers, 1989; Throop & Lerdau, 2004; Evju *et al.*, 2009; Laliberte *et al.*, 2012). As mentioned above, another plant trait which may influence herbivore selectivity is polyploidy (or a larger GS). Genome size is associated with constraints in life history and growth rates (Bennett, 1972; Greilhuber & Leitch, 2013), and may also be impacting C : N : P ratios (Hessen *et al.*, 2010).

There are three types of simultaneous stresses that probably all plants are subject to: resource limitation, competition, and predation. We investigate how GS and ploidy level influence and interact with these three stresses in a long-term grassland experiment established in 1992 (Nash's Field in Silwood Park, UK). We examine the effects of macronutrient (N and P) limitation, herbivore presence (rabbits, insects, molluscs), plant traits (competition, GS and ploidy level), and their interactions, on above-ground plant biomass. We predict that plant communities under grazing pressure are primarily composed of species with smaller GS in contrast to communities in which herbivores are experimentally excluded. This may be because investment in defence may be more costly, and tissue recovery from herbivore damage less efficient in taxa with larger genomes, and/or plants with larger genomes are nutritionally favoured by herbivores.

Methods

Study site

The experimental study was started in 1992 on Nash's Field in Silwood Park, UK (National Grid reference 4 1/944691), an acid mesotrophic grassland on which rabbits (*Oryctolagus cuniculus*) are a keystone species. They have been present at this site since their recovery from myxomatosis in the 1950s, and their grazing has prevented the establishment of woody species (e.g. *Quercus*) and thus the succession from grassland to woodland. The experiment is set up in a split-plot, factorial design with eight blocks, each with one of four +/-insect +/- mollusc treatments. Half of each block is fenced for rabbit exclusion. Within each half-block are pH controlled (limed and unlimed) plots; at the smallest plot level (measuring $2m^2$) are the nutrient treatments, which comprise combinations of: +/- nitrogen (N) as ammonium nitrate, at 100 kg ha^{-1}, +/- phosphate as (P) at 35 kg ha^{-1}, +/- potassium (K), and +/- magnesium (Mg) which are added once a year). In the first three years, herbicide was also applied within each herbivore plot for plant type control ((+/- grass +/- forbs)). Insects are controlled by permethrin synthetic

pyrethroid and Dimethoate-40; molluscs by pellets of metaldehyde; and rabbits by wire mesh fencing (Crawley, 1990; Edwards & Crawley, 1999; Allan & Crawley, 2011). Insecticide and molluscicide are applied three times a year. Small mammals such as field voles (*Microtus agrestis*) and large mammals such as roe deer *(Capreolus capreolus)* are not excluded by rabbit fencing.

Data acquisition

We analysed species mean biomass data collected in 1997 and 2000 from 556 limed $2m^2$ subplots. The experiment contains a total of 576 limed plots, however twenty plots with *Pteridium aquilinum* (bracken, an invasive and toxic fern), were removed from the analyses to focus on angiosperm plant communities. Where possible, species were sampled in 2015 to estimate their C-value using standard flow cytometry methods (Pellicer & Leitch, 2014) with a Partec CyFlow Space flow cytometer fitted with a Cobalt Samba green (532 nm, 100 mW) laser. Internal standards were either *Petroselinum crispum* "Champion Moss Curled" (1C= 2.22 pg), *Pisum sativum* "Minerva Maple" (1C= 4.86 pg), or *Oryza sativa* [1C= 0.5 pg] and samples were prepared with Galbraith's or LB01 buffers. Genome size was estimated for one to eight individuals of 45 species collected from Nash's field (Table S3.1). In total we estimated the 1C-values for 27 species where the flow cytometry peak distribution coefficients of variation were less than five percent; and obtained the remaining 1C-values from the Plant DNA C-values database (Bennett & Leitch, 2012) (Table S3.2). Ploidy level (diploid or polyploidy) of a species was obtained from the Plant DNA C-values database and/or estimated from the Chromosome Counts Database (Rice *et al.*, 2015).

Phylogenetic data

A phylogenetic tree was estimated for the 56 species present in the data to account for non-independent evolutionary relationships among species in the statistical analyses (Fig. S3.1). Nucleotide sequences for the plastid markers matK and rbcL were obtained from Genbank (Benson *et al.*, 2013) (Table S3.3), aligned in MEGA 5.0 (Tamura *et al.*,

2011) using ClustalW, checked visually, and concatenated in SeaView (Gouy *et al.*, 2010). A maximum-likelihood (ML) phylogenetic tree was estimated using the default settings in MEGA, and verified for consistency with the APG IV angiosperm phylogeny (APG IV Group, 2016).

Data analysis

We tested the effect of GS and polyploidy on plant biomass productivity: 1) as traits at the species level, and 2) as properties of the plot (community) level (*sensu* Violle *et al.* (2007)). We refer to GS as the 1C-value (the amount of DNA in the unreplicated haploid nucleus) in picograms (pg), where 1 pg = 978 Mbp (Dolezel *et al.*, 2003). Statistical analyses were carried out in R version 3.2.1 (R Development Core Team, 2012).

Species level analyses

We first analysed the effect of GS, polyploidy, herbivore exclusion, and nutrient treatment on plant biomass at the species level with a phylogenetic generalised linear mixed model (PGLMM) with Bayesian estimation, by fitting Markov chain Monte Carlo generalised linear mixed models (MCMCglmm) from the R package *MCMCglmm* (Hadfield 2010). The presence of insects, molluscs, rabbits, and the application of N and P fertilizer were scored as binary variables (+/- insecticide, +/-pellets, +/- fencing, +/-N, +/-P). Ploidy level was also scored as a binary variable, where a species was considered either as diploid or polyploid. Evolutionary non-independence was controlled for by specifying a correlation matrix estimated from the phylogeny with the *inverseA* function from the same R package. Block, phylogeny and individual species effects were treated as random effects. We first tested five-way interactions between each herbivore, GS, ploidy, N, and P. Phosphate showed no significant interactions with GS or herbivory, consequently, models were re-fitted without P interactions. We used priors where nu=0.002 and variance=1 and ran the model with ten million generations including a burn-in of 10,000 and a thinning interval of 1000. The *idh* function was used to allow different variances within each herbivore guild. Model convergence was examined with

trace plots, autocorrelation plots, and Heidelberg and Welch diagnostics (Plummer *et al.*, 2010).

Community level analyses

We then investigated the effects of GS, nutrients, and herbivory at the community level. Each plot is representative of a plant community growing under various combinations of nutrient availability and herbivore guilds. For each of the 556 plots, biomass-weighted mean GS was estimated with the phylogenetic generalized least squares method (PGLS). We applied the *gls* function in the 'nlme' R package (Pinheiro *et al.*, 2013), and with the 'ape' package (Paradis *et al.*, 2004) to account for phylogenetic relatedness. We fitted regressions with a Brownian motion correlation structure derived from the phylogenetic tree, and maintained the same phylogenetic correlation structure across all plots. Species percent biomass was used as weighting. Henceforth we refer to the percent biomass-weighted mean GS in which phylogeny was accounted for as 'weighted mean GS'. To assess whether weighted mean GS is a function of herbivory and nutrient application, we fitted linear mixed effect (LME) models (Pinheiro *et al.*, 2013; Bates *et al.*, 2014) where each herbivore type (insects, molluscs, rabbits) and each nutrient (N, P, K, Mg), were scored as binary factors, and block treated as random effect. Interactions between these and the significance of each factor were tested using maximum likelihood (ML) stepwise model reduction to estimate the most parsimonious best-fit model. Model terms associated with a decrease in the Akaike information criterion (AIC) were retained and tested in the next most reduced model. The most reduced model was refitted with restricted ML (ReML).

Confirmatory Path Analysis

We examined the effects of herbivory, N, and P on plant community structure using confirmatory path analysis, focussing on rabbits and molluscs. Data were partitioned to include: 1) control and + rabbit plots on which invertebrate and molluscs were excluded, for a total of 62 plots, and 2) control and + molluscs plots on which insects and rabbits

were excluded, for a total of 68 plots. In addition to weighted mean GS, we also examine, at the community level, how polyploidy, plant resistance to herbivore grazing, and plant competitiveness interact and potentially exert effects on each other and total plot biomass under herbivory and nutrient treatment. We excluded plots fertilized with potassium and focussed on the effects of N and P. A species was evaluated as herbivore-resistant, when its relative contribution to total plot biomass increased in the presence of the herbivore, either with or without N fertilizer treatment. Twenty-two species were evaluated as rabbit resistant, the most important being *Holcus lanatus* and *Jacobaea vulgaris*, and nineteen as resistant to mollusc herbivory, in particular *Festuca rubra* (Table S3.4).

More specifically, five community properties were assessed: (i) weighted mean GS; (ii) polyploid abundance (i.e. the percentage of the total biomass that arises from polyploidy taxa); (iii) grazing resistance (i.e. the abundance (percentage of the total biomass) that arises from herbivore-resistant species); (iv) mean competition, as defined in Grime's C-S-R plant strategy framework (Grime, 1977), which describes taxa as either, or a combination of, three strategies: competitor (C), stress tolerant (S), and ruderal (R) on a scale of zero to one. Each taxon's C-strategy was attributed following Hodgson et al. (1999). Mean competition of each plot was estimated with PGLS, as described above, for weighted mean GS. The fifth community property (v), is total plant biomass (i.e. the total dry weight of the plot).

We used directional separation (*d-sep*) path analysis methods (Shipley, 2009) to assess seventeen hypotheses about how these plant traits are linked with each other and are influenced by herbivory and nutrient (N and P) availability (Fig. S3.2). These hypotheses, formulated as directed acyclic diagrams, are built upon previous findings on the influence of GS and polyploidy on plant communities growing under macronutrient limitation and from examining correlations present in the data (see also Table S3.6 for a more complete description of *d-sep* methods).

Path analysis is used here to assess plausible explanations for the effect of the experimental treatment (herbivory exclusion, N and P fertilizer) on the five plant community properties. The conditional independencies were fitted with generalised least squares (GLS). Continuous predictor variables were standardized by two standard deviations (Gelman, 2008) with the rescale function from the 'arm' package (Gelman *et al.*, 2016). Each GLS regression was systematically fitted with ten unequal variance structures using the *varIdent* function (Pinheiro *et al.*, 2013), to account for heteroscedasticity in the residuals (Table S3.6) (Zuur *et al.*, 2009). We retained the p-value from the regression resulting in the lowest second-order AIC (AIC*c*), which corrects for small sample sizes, implemented in the package 'AICcmodavg' (Mazerolle, 2016), but only if an analysis of variance showed that a regression fitted with the variance structure was significantly better ($p < 0.05$) than a regression fitted without. We also included block as a fixed effect when fitting the rabbit equations. Interactions among community properties, and between the experiment and community properties, were not allowed. We present the models with the lowest CICc-statistic for each dataset (rabbits, molluscs) (Table S3.7). We based our selection on CICc because it favoured the simpler more parsimonious path models, but this does not exclude alternative or more complex hypotheses that passed the goodness of fit tests.

Due to the complexity of the interactions under different herbivory and N regimes, we also show relationships between community plant traits from the herbivore guilds investigated above with +/- N in scatter plots and the adj. R^2 value obtained by a simple linear analyses here and in the SI. We included functional group (specifically grasses, species belonging to the taxonomic order Poales) as a sixth trait in the SI, to better understand whether and how functional group provides insight on plant communities at Nash's field.

A path analysis of plant communities living within fenced plots (i.e. no rabbits) is provided in the supplementary information (n=68 plots). These represent natural

grassland communities including their insect and mollusc guilds, and without the impacts of rabbits, which is a non-native mammal in the British Isles. We do not provide a separate path analysis on the effects of insects only, because this is a complex and very diverse group which includes not only different types of herbivory, but also mutually beneficial species as well.

Results

Species biomass is a function of interactions between genomic parameters, nutrients, and herbivory

Using PGLMM, we show that associations between genomic parameters (GS and ploidy), biomass, and grazing pressure are most pronounced on plots with rabbits (Fig. 3.1a, Table S3.8). As expected, biomass production increases significantly with N, on average 2.25-fold (B= 0.522, pMCMC = 0.0078) in comparison with control plots (B= 0.232). The effects of N and herbivory are moderated by the GS of a species and whether it is diploid or polyploid. The effect of P on species biomass is weaker, increasing only 0.55-fold on average (B= 0.128, pMCMC= 0.0273). Phosphate was not found to have any significant interactions with model parameters in the preliminary analyses on species biomass, and so we refitted the PGLMM without P interactions.

Complex four-way, three-way, and two-way interactions between GS, ploidy, N and herbivore type impact species biomass productivity. The effects of interactions between molluscs, GS, ploidy and N result in significant increases in biomass (Fig. 3.1a, Table S3.8). On plots with applied N, mollusc herbivory results in more polyploid taxa with larger GS (Fig. 3.1a). In contrast, the opposite is true of rabbit grazing, where interactions between rabbit grazing, GS or ploidy and N result in a decrease in biomass (Table S3.8), with rabbit-grazing predominantly decreasing biomass production from polyploid plants with a large GS (Fig. 3.1a, Table S3.8).

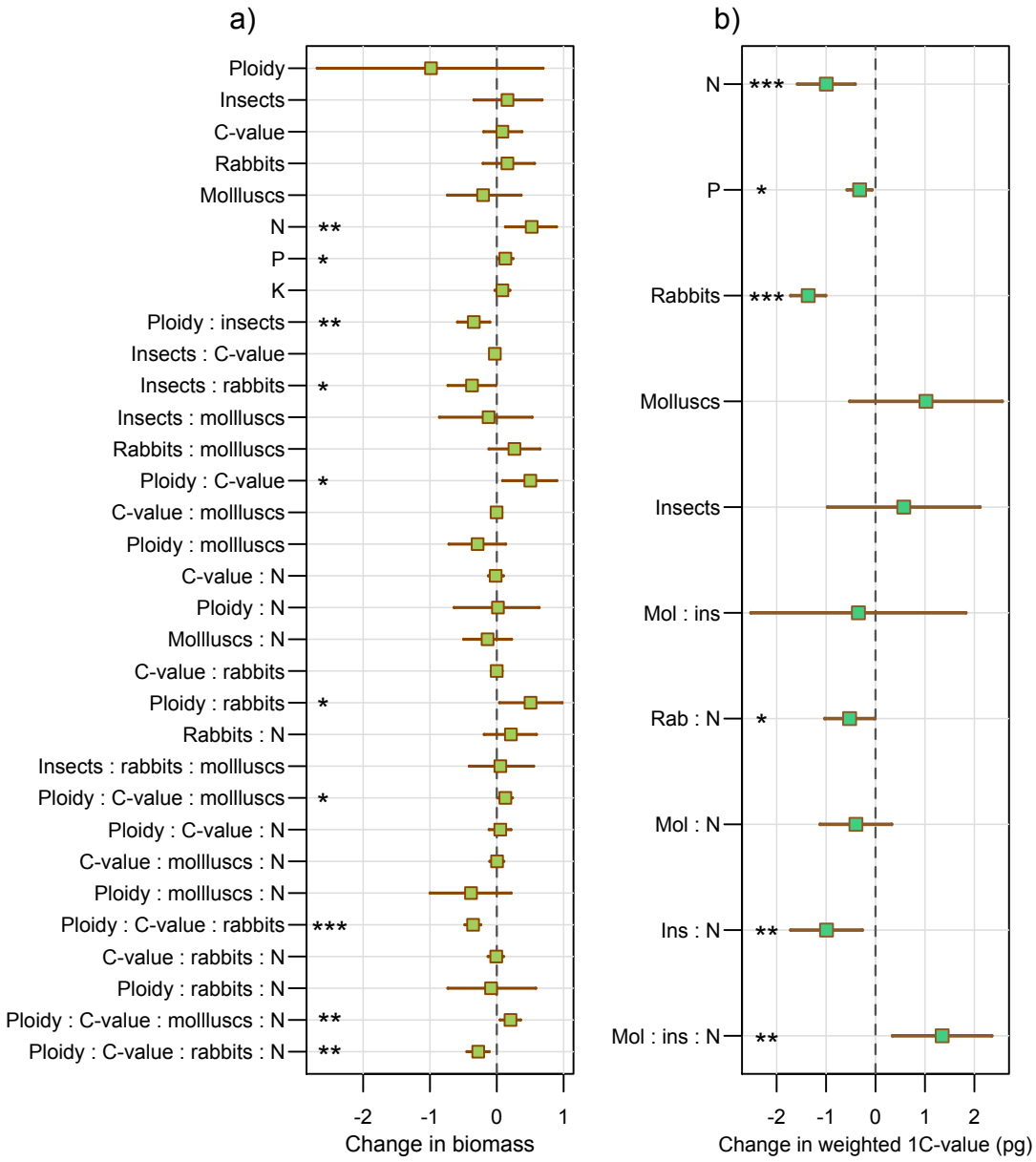

Figure 3. 1 a) PGLMM: Species biomass as a function of GS (C-value), ploidy level, herbivore treatment (rabbits, molluscs, insects), and nutrient input (N, P, K). The intercept = 0.232, with 95% confidence intervals (CI) of-3.73 and 4.40. See Table S3.8 for full model output. **b**) LME: Effects of experimental treatment (herbivore exclusion and nitrogen (N) and phosphate (P) input) on biomass-weighted mean GS. The intercept coefficient = 5.704, with 95% CI =4.90, 6.51. The most parsimonious LME model shows a significant increase in mean GS with a three-way effect of N, molluscs, and insects; conversely the combined effect of N and rabbit grazing shows a decrease in mean GS. P-values are represented as follows: *** < 0.0001, ** < 0.001, * < 0.05. See also tables S3.8

Weighted mean GS of plots is a function of nutrients and herbivory

Community (i.e. plot) weighted mean GS were estimated for each of the 556 plots and fitted in a LME model as a function of herbivore and N treatment. Mean GS of the plots ranged from 1.36 pg (+N; + insects) to 8.65 pg (+N; minus rabbits). Plant communities of plots characterised by small weighted mean GS tend to be dominated by *H. lanatus*, whereas those with a large mean GS are mainly dominated by *Arrhenatherum elatius*. Following model reduction methods (Table S3.9), the most notable effect on plot biomass-weighted mean GS is the presence of rabbits. (Fig. 3.1b). Rabbits significantly reduce plot weighted mean GS, especially in the presence of N, from an estimated mean of 5.70 pg on control plots, to 2.82 pg (Fig. 3.1b, Fig. 3.2, Table S3.10). The largest weighted mean GS are found in plant communities where insects and molluscs are present and in which rabbits are excluded, and which are fertilised with N, although it is also these plant communities that show the largest variation in mean GS (Table S3.11 for standard deviations). Nitrogen treatment leads to a highly significant increase in total plot biomass ($B = 42.90$, $F(1, 538) = 214.05$, $p < 0.0001$) and a decrease in species numbers ($B = -0.29$, df =553, residual deviance= 550.77, $p < 0.0001$) (Fig. S3.3, Table S3.12).

Plant community properties on rabbit plots

We used path analysis to untangle the effects of herbivory and nutrient treatment on polyploid abundance, mean GS, competition, plant resistance to herbivory, and total plot biomass. Weighted mean GS and mean competition are proxies for the mean GS and the competiveness (C-strategy) of each plant within the plot community. The presence of rabbits has the biggest effect on weighted mean GS, which decreases by 2.12 pg ($p < 0.0001$). Nitrogen input, perhaps surprisingly also has a negative effect on weighted GS (-1.76 pg, $p = 0.0032$) (Fig. 3.3, Table S3.13i). The negative association between rabbits, and GS is also shown by the PGLMM and LME above. Plant competitiveness is most

influenced by the availability of N and P, rather than rabbit presence (Fig. 3.3). On

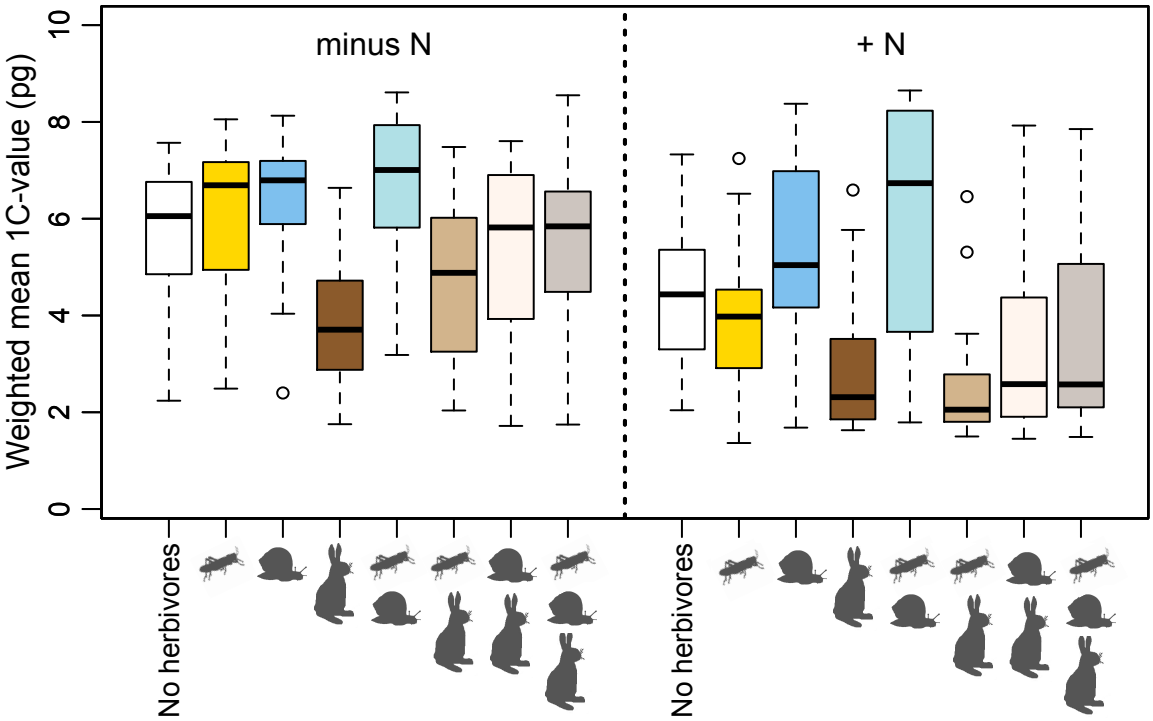

Figure 3. 2 Biomass-weighted mean GS (estimated with phylogenetic GLS), of each plot under eight different herbivore exclusion treatments and +/- nitrogen (N) input. Herbivore treatments, in order as shown below are: 1) no herbivores (control); 2) + insects; 3) + molluscs; 4) + rabbits; 5) + insects + molluscs; 6) + insects + rabbits; 7) + molluscs + rabbits; 8) all herbivores (untreated and unfenced plots). Mean GS decreases significantly in plant communities with rabbits whilst increasing with insects + molluscs (see also Fig. 3.1). Boxes show median weighted mean GS, first and third quartiles, and minimum and maximum.

control plots, the abundance of rabbit-resistant plants show a highly significant positive

association with mean competition (control – N: adj. R^2= 0.666, p< 0.0001; control + N: adj. R^2 = 0.408, p = 0.0046). The presence of rabbits disrupts this correlation with competition entirely (Fig. S3.4 a-d). The strongest predictor of rabbit-resistance is GS (Fig. 3.3), where communities with a high proportion of rabbit-resistant biomass contain a higher proportion of species with smaller GS. This is most seen where plants are

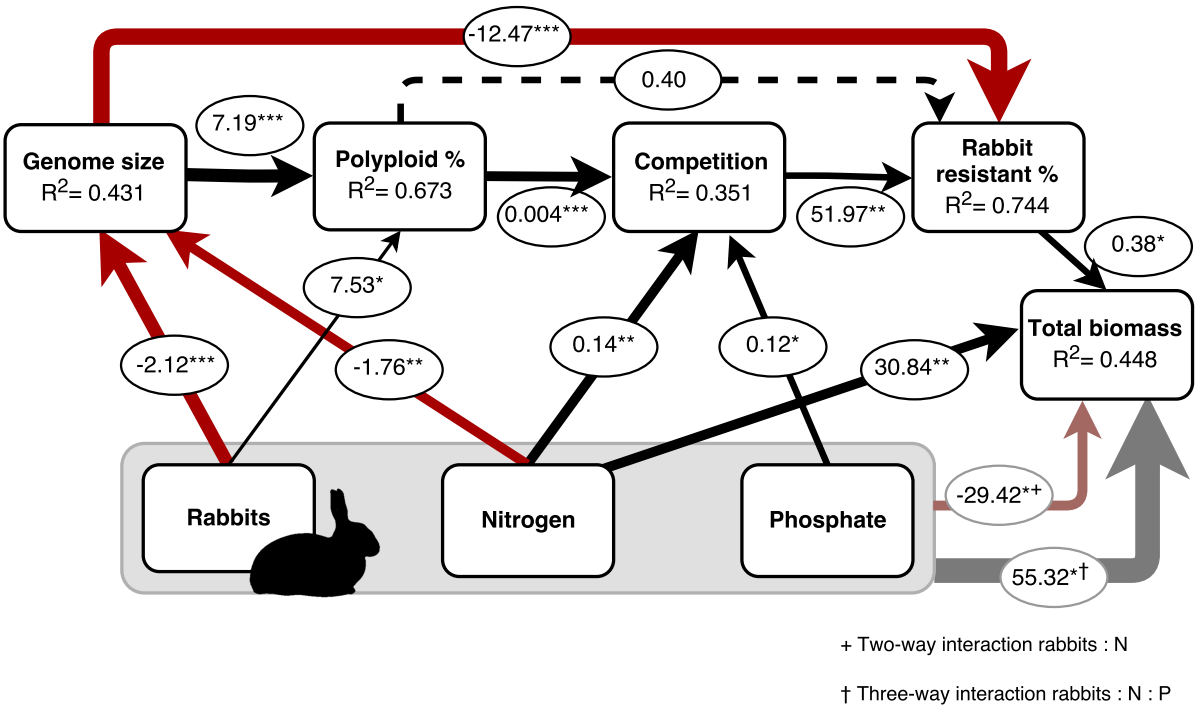

Figure 3. 3 Path analysis is used to disentangle effects of rabbits, nitrogen and phosphate on five plant community traits: biomass-weighted mean genome size (estimated with phylogenetic GLS), percent biomass of polyploid plants, biomass-weighted mean competition (estimated with phylogenetic GLS), percent biomass of species resistant to rabbit grazing, and total plot biomass. Values shown are partial regression coefficients: the amount a dependent variable increases for a one-unit increase in an independent variable, holding all other independent variables constant. The experimental treatment parameters were treated as binary factors (+/- N, +/- P, +/- herbivore) to obtain a coefficient for each effect. Arrow widths are based on the standardised regression coefficient (to two standard deviations), which gives a representation of the relative effect of each parameter. The adjusted R^2 is given for each trait, as a measure of how much variation is explained by the independent variables, obtained from a simple linear regression. Positive (increasing) effects are shown in black, negative (decreasing) effects are shown in red. Non-significant effects of the experimental treatment are not displayed; a dashed arrow indicates a non-significant effect among the community traits. See also Table S3.12 for full details on each path regression. P-values are represented as follows: *** < 0.0001, ** < 0.001, * < 0.05.

growing on N-limited plots (Fig. 3.4 a-d); this correlation is absent on plots with conditions of least stress (N input and herbivore exclusion) (Fig. 3.4b). The effect of polyploidy on mean competition also depends on whether N is a limiting factor.

Competitive plant species were shown to be predominantly polyploids growing on N fertilized plots ($F(1, 29) = 16.38$, $p = 0.0004$, adjusted $R^2 = 0.339$) (Table S3.13b, Fig. 3.2). In contrast, polyploids that occur on plots with N limitation tend to have low C-strategy scores, especially on plots with rabbits ($F(1, 13) = 15.4$, $p = 0.0153$, adj $R^2 = 0.5164$) (Fig. S3.5 a,b,d,e). Polyploid biomass and weighted mean GS are positively correlated across all treatments, and this is most pronounced on rabbit-grazed plots with added N (Fig. 3.5). Among the parameters included in the model, the principal driver of total biomass production in plant communities is a three-way interaction between rabbits, N and P (Fig. 3.3, Table S3.13e, $B = 55.32$, $F(9, 52) = 6.49$, $p = 0.0224$).

Contrasting and similar patterns with mollusc herbivory

Plant communities growing on plots with grazing pressure from molluscs differ in three main ways from those with rabbit grazing. First, the association between weighted mean GS and percent of mollusc-resistant biomass is positive, (Fig. 3.6). This association, where more plants with larger genomes correlate with an increase in plants that tolerate mollusc grazing, is strongest in communities growing on + molluscs and + N plots (Fig. 3.4e-h). This is the inverse relationship to that seen with rabbits (Fig. 3.4a-d). Secondly, a high abundance of mollusc-resistant species correlates with low mean competition (Fig. S3.4e-h). This is also the inverse relationship to that seen in rabbit-resistant biomass. Third, an increasing abundance of polyploids is associated with increased competition on mollusc-grazed plots which are N-limited (Fig. S3.5c), in contrast to + rabbits, –N plots (Fig. S3.5b). Another difference is that a three-way interaction between molluscs, N and P decreases biomass-weighted GS ($F(7, 60) = 4.188$, adj. $R^2 = 0.250$, $p = 0.0008$, Table S3.13ii).

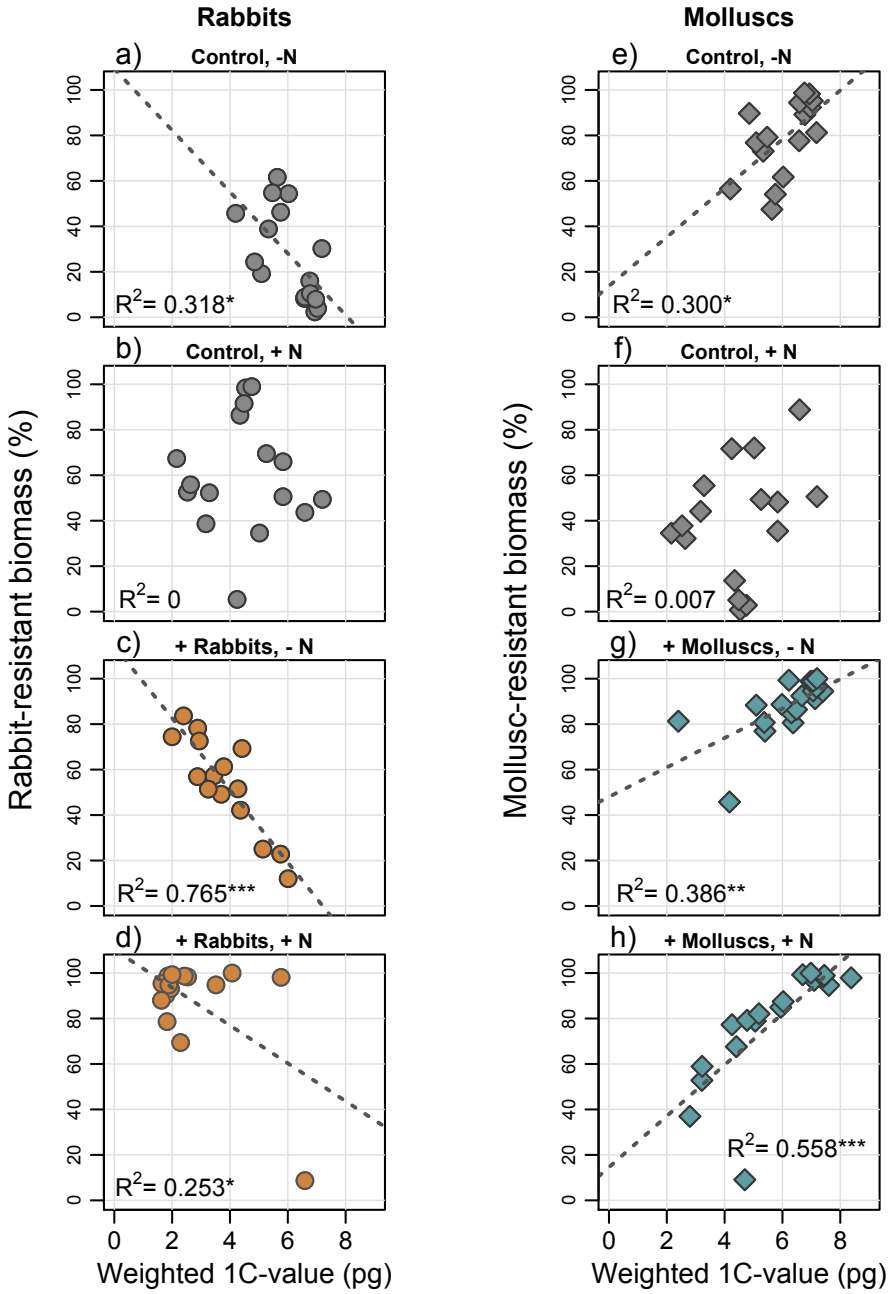

Figure 3. 4 Relationships between weighted mean GS and percent biomass of: **a**) -**d**) rabbit-resistant species; and **e**)-**h**) mollusc-resistant species; under four different experimental treatments: 1) control (rabbit, mollusc, and insect excluded plots) minus nitrogen (-N); 2) control + N; 3) + herbivore (rabbits (**c**, **d**), or molluscs (**g**, **h**)) minus N; 4) + herbivore + N. The adjusted R^2 is shown in each plot. Rabbits: **a**) B = -13.54, F(1, 14) = 7.98, p= 0.0135; **b**) B = -0.30, F(1, 14) = 0.004, p= 0.95; **c**) B = -15.87, F(1, 13) = 46.70, p < 0.0001; **d**) B = -8.33, F(1, 13) = 5.75, p = 0.0322. Molluscs: **e**) B = 10.76, F(1, 14) = 7.42, p= 0.0165; **f**) B = 4.72, F(1, 14) = 1.10, p= 0.3121; **g**) B= 6.48, F(1, 16) = 11.67, p < 0.0035; **h**) B= 11.25, F(1, 16) = 22.43, p = 0.0002.

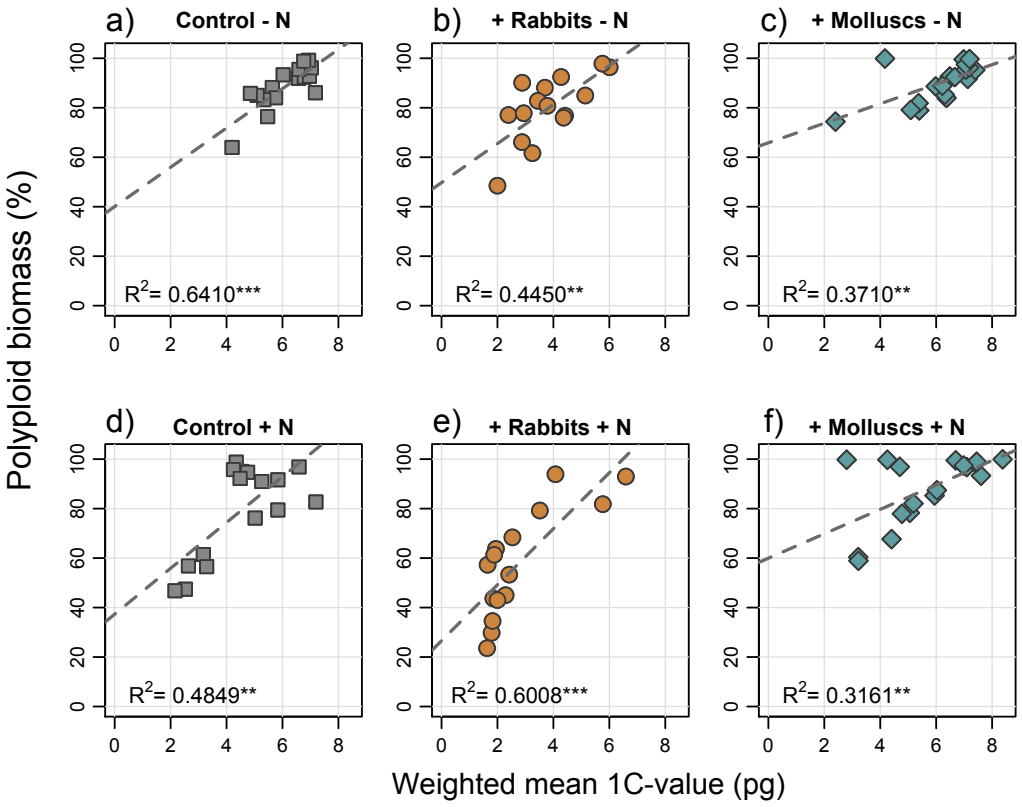

Figure 3. 5 Associations between biomass-weighted mean GS and percent polyploid biomass on: **a**), **d**) control plots (plots where insects, molluscs, and rabbits are excluded; **b**), **d**) + rabbit plots; and c), f) + mollusc plots; each with -/+ N. The adjusted R is shown in each plot. Control -N: **a)** B = 7.94, F(1, 14) = 24.87, p= 0.0002; **b)** B = 7.88, F(1, 13) = 12.23, p= 0.0039; **c)** B = 3.95, F(1, 16) = 11.03, p < 0.0043; **d)** B = 9.26, F(1, 14) = 15.12, p = 0.0016. Molluscs: **e)** B = 11.32, F(1, 13) = 22.07, p= 0.0004; **f)**) B= 4.93, F(1, 16) = 8.86, p = 0.0089.

There are two main similarities between the mollusc and rabbit plots. For both herbivores, weighted GS is the main predictor of polyploid abundance, more so than nutrient availability or grazing pressure (Fig. 3.5, 3.6 Table S3.13ii). Second, N has a direct, negative effect on biomass-weighted mean GS.

A significant positive relationship between competition and GS with N input, as hypothesised, was only found in plant communities where all herbivores were excluded (F(1, 14)= 6.56, p = 0.0226, Fig. S3.6d). We found that plant functional group,

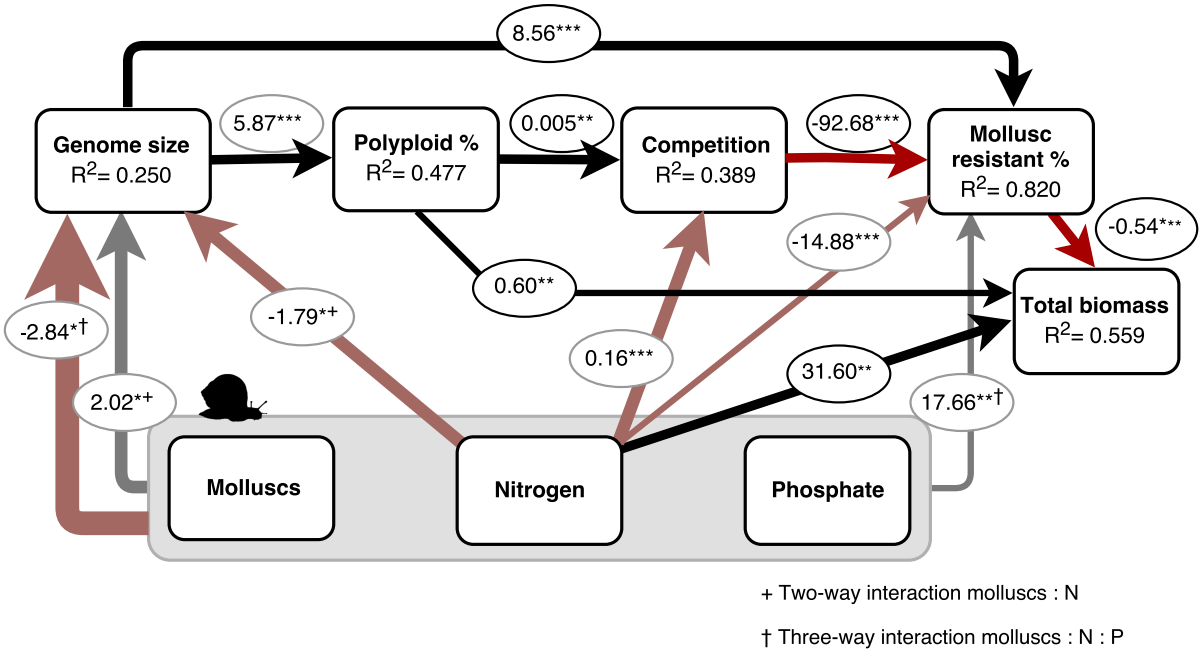

Figure 3. 6 Path analysis unpicking effects of molluscs, nitrogen and phosphate on five plant community traits: biomass-weighted mean GS, percent biomass of polyploid plants, mean competition (estimated with phylogenetic GLS), percent biomass of species resistant to rabbit grazing, and total plot biomass. The adjusted R^2 is shown for each plant community trait. Positive (increasing) effects are shown in black, negative (decreasing) effects are shown in red.

specifically graminoids, was not as informative in predicting community abundance of herbivore-resistant plants as GS (Fig. S3.7), although correlations can be seen between weighted mean GS and graminoids on +rabbit -N plots in particular (R^2= 0.377, p= 0.0088) (Fig. S3.8b). Grass abundance shows no, or very little, association with polyploid abundance (Fig. S3.9) and with mean competition (Fig. S3.10).

We also present path analysis on plant communities with both insects and molluscs but which are not subject to rabbit grazing (i.e. fenced plots without pesticides) in Figs S3.11- S3.14. Very briefly, we find a positive effect of GS on the biomass of herbivore-resistant species, especially on the control plots without N input (adj. R^2 = 0.387, p= 0.006) (Fig. S3.12, Table S3.13iii). In contrast to communities with rabbit-only and mollusc-only grazing, path analysis shows a negative association between polyploid

abundance and abundance of mollusc and insect-resistant plants (B= -0.97, $p < 0.0001$) (Fig. S3.11). Similar to results reported above, polyploid abundance is closely linked with mean GS ($R^2 = 0.943$, $p < 0.0001$ on +N plots) (Fig. S3.13f). With N input, significant positive relationships are found between GS and competition (adj. $R^2 = 0.802$, $p < 0.0001$) (Fig. S3.13d), and between polyploid abundance and competition (adj. $R^2 = 0.668$, $p < 0.0001$) (Fig. S3.13e). Combined, these results indicate that the genomic parameters (GS and polyploidy) play a role in defining plant community structure, and are under the direct effect of rabbit and invertebrate herbivory.

Discussion

Our study does indeed suggest that GS is an essential, yet missing link, in our understanding of the cascade of N and P through the ecosystem. Previously, research from Park Grass and Rengen field trials pointed to a role for plant GS in the generation of biomass, dependent on availability of N and P. Specifically, the most marked effect being that competitive polyploids with large GS contributed most to biomass when N and P were both present. We were, however, unable to observe a similar effect from the joint application of N and P fertiliser on GS at Nash's field site, perhaps because the experiment is younger (data was collected when the experiment was only five and eight years old, compared with 160 years for Park Grass and 75 for Rengen), and plant communities may still be adapting to the imposed experimental treatments and are in a transient state (Lehman & Tilman, 2000). In a similar experiment with fertiliser and sheep grazing (Laliberte *et al.*, 2012), fertiliser treatment caused a rapid, positive response in most traits over the first four to five years, followed by rapid change in direction of the trait responses and subsequent fluctuations, before reaching a relatively stable state with fewer fluctuations after 19 years; during which differences in sheep grazing intensity were subtle and became more apparent after c. 15 years. Nevertheless,

at Nash's Field there were significant effects of interactions between herbivory, N application and GS on the generation of biomass which we explore below.

Do herbivores select plants dependent on their GS?

Plants that increase in biomass in the presence of herbivores may be opportunists, fast-growing competitors that are able to compensate for damaged parts, or are not being eaten by herbivores, the latter which could be due to palatability or herbivore preference. We find a strong negative association between mean weighted GS and rabbit grazing. This association is highly significant when analysed using PGLMMs and LME models. Furthermore PGLMMs show that plant abundances are not affected by rabbit grazing and nutrient availability alone, but that the effect of herbivory on plant productivity also depends on GS and ploidy level. Thus these two genomic parameters may be influencing rabbit selection within a stoichiometric context, and/or are influencing plant and community responses to grazing pressure. The negative correlations observed between rabbit-resistant species biomass and GS, which intensified on + rabbit plots, may be due to different processes as rabbits impact the plant communities through direct consumption and the local habitat through disturbances. These activities may lead to local extinction, relaxed plant competition, and allow colonization of functionally more diverse species into the community (Olff & Ritchie, 1998). Genome size and polyploidy may be playing a role in some or all these processes, enabling or inhibiting species establishment, capacity for regrowth, and ability to compete for different resources, in particular macronutrients.

Keeping in mind that grassland dynamics are complex, species which increase in biomass after herbivore exclusion may indeed be indicative of species that are preferentially grazed on by the herbivores (Kempel *et al.*, 2015). A possible contributory factor to the increased weighted mean GS of plots in the absence of rabbit grazing may be rabbit preference for species with larger GS. One reason why rabbits (and perhaps insects too) are selecting plants based on GS is because they may be of higher nutrient quality,

and rabbits are known to favour high-nutrient plants (Miller, 1968). Plants with big genomes have more nucleic acids and proteins to pack into chromatin per cell. DNA and RNA are amongst the most N and P rich biomolecules and histones, being N rich, are amongst the most abundant proteins of the cell, and fast-growing plants are likely to have higher RNA : DNA ratios. Thus chromatin is demanding for these essential macronutrients and potentially chromatin-rich cells are more nutritious for rabbits. Conversely, molluscs show a compensatory effect, where communities with a large weighted mean GS contain a proportionally large amount of mollusc-resistant biomass. These are plots that tend to be composed primarily of polyploid grasses *Festuca rubra* and *Arrhenatherum elatius*, whereas species that decreased the most with mollusc herbivory are *Holcus mollis*, also a polyploid grass, followed by *Achillea millefolium* (Asteraceae), a forb with a large GS and *Holcus lanatus*, a diploid grass with a small GS. Although generalist herbivores, feeding trials have shown molluscs prefer some species over others (Dirzo, 1980), and different mollusc species show different preferences. As a guild, mollusc herbivores appear to have a mitigating effect on rabbit herbivory, perhaps because they are specialised to feed on fast-growing taxa which put little resources into the production of secondary metabolites (Fraser & Grime, 1999), or their preferences are based on plant morphology. We recommend mollusc feeding preference trials where variation in polyploid cytoypes are among feeding choices.

Nitrogen input has a strong effect on plant biomass and community structure. It is known that soil nutrient availability can qualitatively change plant nutrient content (Güsewell, 2004) and quantitatively alter biomass production. Plants growing under higher N may become more attractive to consumers and increase herbivore numbers (Ball *et al.*, 2000; Nevo & Coll, 2001), but as noted above, higher food quality may reduce per capita consumption. The interplay between increased plant productivity under fertilizer regimes would lead to increased competition among fast growing species (Grime, 1977). The significant three-way interactions between rabbit herbivory, GS and N input in the production of biomass could be because rabbits can generate a fast and

sustained stress on the plant community. Path analysis shows GS remains a key trait influencing feed-back mechanisms between grazing pressure and N, influencing functional group and competition.

Multiple interactions between resource availability, plant traits, and plant consumers are further influenced by many other factors. These include traits of the herbivore themselves, which may vary with nutrient acquisition strategies (Deraison *et al.*, 2015), and the scale and impact of herbivores which can be linked with their size (Brown *et al.*, 2004; Schramski *et al.*, 2015). Other processes that may be influencing the dynamics between plants and the animals that feed on them are past history of herbivory (Howe & Brown, 2001; Rasmann *et al.*, 2012), evolutionary history of the grazing site (Milchunas *et al.*, 1988) different induced responses in different plant types, which are also dependent on soil nutrients (Karban & Myers, 1989), season (Bryant *et al.*, 1983; Bullock *et al.*, 2001; Pardo *et al.*, 2015), genetic variation within a species (Barbour *et al.*, 2015), and multiple levels within a herbivore guild (Ohgushi, 2005).

Nitrogen and/or P availability have direct ecological impacts throughout food webs, and when in excess can severely impact ecosystem functioning. A key challenge we face into the future is to increase yield whilst maintaining biodiversity - a challenge that is global and likely to become more acute as the human population grows. Thus it is necessary to more fully understand ecosystem services, one of which is nutrient cycling, which is dependent on interactions between plants and herbivores. Plant traits important to nutrient cycling include C, N, and P foliage contents; when eaten by animals, these elements are partly returned to the environment as waste products, which are then more or less rapidly decomposed, depending on the elemental quality (Olff & Ritchie, 1998; van Wijnen *et al.*, 1999; Belovsky & Slade, 2000). Investigations on plant traits have uncovered global trends that help to predict plant responses and effects (Reich & Oleksyn, 2004) to abiotic and biotic factors. Much remains to be studied if we are to fully understand the direct and indirect effects of GS and ploidy level within ecological

systems and how they influence such ecosystem services as nutrient cycling - effects which potentially have far-reaching impacts.

Chapter 4 Angiosperm species realised niche attributes are influenced by genome size and ploidy level

This chapter is formatted for submission, however the authors and the journal are still undecided.

Summary

Angiosperm species occupy a wide range of habitats that vary in the levels of light, water and nutrients that are available. These resources partly comprise the abiotic realised niche of a species, the environmental space that a species occupies. We hypothesise that genome size may play a role in determining a species' realised niche, by influencing its ability to compete for, and cope with the full range of these resources, in particular nutrient availability. We examine the associations between Ellenberg's indicator values of species preferences for nitrogen, light, and water; and Grime's competitor strategy, which is an estimate of a species' ability to compete for resources; with published genome size data. We use regression and principal component methods within a phylogenetic framework. We report significant effects of genome size and polyploidy on species values for nitrogen (nutrients) and light.

Introduction

Angiosperm genome sizes (GS) range an astonishing c. 2,400 fold, from the smallest recorded genome in the carnivorous plant *Genlisea tuberosa* (Lentibulariaceae, 1C-value = 0.059pg, or 61Mbp) (Fleischmann *et al.*, 2014), to the largest known eukaryote genome in *Paris japonica* (Melanthiaceae, 1C = 152.23 pg) (Pellicer *et al.*, 2010). Angiosperm genomes exhibit extreme plasticity with an estimated in 20% to 70% of species which are polyploidy (depending on the method, Husband *et al.*, 2013) and the genomic signatures of multiple rounds of polyploidy in their evolutionary history (Van de Peer *et al.*, 2009; Jiao *et al.*, 2011; Fawcett *et al.*, 2013). Whole genome duplication is frequently followed by genome downsizing (Leitch & Bennett, 2004), and this may contribute to the observation that most angiosperms are characterised by possessing small genomes (mean 1C-value = 5.48 pg, median = 1.93 pg, mode = 0.6 pg, and mode rounded to nearest whole number = 1pg, n = 8881 (based on data available in Bennett & Leitch (2012) and additional C-values not yet incorporated into the database).

The wide range in angiosperm GS has been shown to have ecological implications and potential trade-offs. Species with large genomes may be excluded from particular life strategies (Bennett, 1987), extreme ecological niches (Knight & Ackerly, 2002), are more prone to being excluded from polluted sites (Vidic *et al.*, 2009; Temsch *et al.*, 2010), and are at higher risk of extinction (Vinogradov, 2003). Such correlations likely arise, in part, from constraints imposed by large genomes at the cellular level (Greilhuber & Leitch 2013). Further constraints of GS may also become apparent when nutrient availability is limited (Leitch & Bennett, 2004; Hessen *et al.*, 2010). For example, two recent studies on long term grassland field plots differing in nutrient levels have shown that species with larger genomes are less productive on soils with low nutrients, but when they are released from nutrient limitation many of these species outcompete taxa with smaller GS (Šmarda *et al.*, 2013; Guignard *et al.*, 2016). Coupled with GS is ploidy level, where higher ploidy levels generally lead to a larger GS (diploid median 1C-value = 1.89,

polyploid mean 1C-value = 3.46, n= 4368 and 1971 respectively). These two traits influence each other in different ways. At the cellular level a chromosome doubling increases GS, however at the ecological level the abundance of a diploid or a polyploid species can in turn be influenced by GS (Šmarda *et al.* 2013; Guignard *et al.* 2016). Polyploidy has been associated with invasiveness (te Beest *et al.*, 2012), the ability to establish in new ecological niches (see Ramsey & Ramsey 2014 for a review), and increased resistance to stress conditions such as aridity (Manzaneda *et al.*, 2012; Diallo *et al.*, 2016). Such advantages may be due to duplicated genes assuming new functions (i.e. neofunctionalization) or acquiring a secondary function (i.e subfunctionalization) (Oh *et al.*, 2012). Allopolyploidy (whole genome duplication between different species) is also a key factor in promoting the robustness, high yield, and larger sizes of most the world's crop plots (Renny-Byfield & Wendel, 2014).

We propose here that GS and ploidy level are genomic traits that influence the realized niche of a species, (i.e. the actual ecological space occupied, comprising both abiotic and biotic attributes), particularly in terms of nutrient availability. Nucleic acids are by mass approximately 39% N and 9% P (assuming an equal ratio of purines and pyrimidines), these are relatively high proportions compared with other organic molecules, and hence are costly to synthesise and express (Sterner & Elser, 2002; Hessen *et al.*, 2010). With such a large variation in GS within angiosperms, it seems likely that the cellular demands for these elements will vary enormously between species. In many environments N and P are limiting to plant growth (Vitousek *et al.*, 2010; Nussaume *et al.*, 2011) and potentially may offer a selection pressure against large GS in certain circumstances (Bragg & Wagner, 2009; Greilhuber & Leitch, 2013). A comparative analysis of the nucleotide and amino acid compositions of genes and their proteins in a small sample of animals and plants showed that wild plant species had low N-demanding nucleotides and amino acids. The analysed crop plants, which have a history of fertilizer application, and animals, which are heterotrophs harvesting N from other organisms, showed no evidence of N-selection in their transcriptomes and proteomes (Acquisti *et*

al., 2009a, b). These studies, together with the grassland experiments mentioned above, are suggestive of selection for species with reduced GS imposed by nutrient availability. Similarly in the freshwater snail *Potamopyrgus antipodarum,* triploid variants showed a two-fold increase in growth rate compared with the tetraploids when fed a low P diet, indicative of selection pressures mediated by phosphate availability on GS (Neiman *et al.*, 2013).

Given the above, which suggests that nutrient availability does indeed impose a selection pressure on GS and thus GS may influence the niche a plant occupies. We aimed to determine the extent to which a taxon's realised niche is influenced by its GS and ploidy. To achieve this, we have exploited available data on plant ecological attributes, established by Ellenberg *et al.* (Ellenberg, 1974; Ellenberg *et al.*, 1992), who report indicator values for 1791 plant taxa scored on arbitrary ordinal scales (for a definition of the scores, see Table 4.1). These scores represent a taxon's realised niche. Ellenberg's N which initially stood for nitrogen, is an estimation of the soil fertility niche (Hill *et al.*, 2004), and by extension reflects nutrient availability. Within a stoichiometric framework, it is the most obvious direct link to GS. In a multi-dimensional habitat space environmental stresses co-occur simultaneously, yet most plants cannot tolerate more than one type of severe stress (Mittler, 2006). The effect of stress from one environmental factor is often increased when accompanied by stress from another factor (e.g. light damage is aggravated by water limitation (Powles, 1984)). A plant's resource requirements also include solar radiation and water, and a high demand for one resource may necessitate a high demand for another, and/or alternatively be compensated by a lower demand for a second or third resource. For example, the level of rubisco, the most important CO_2-fixing enzyme, is simultaneously controlled by levels of N, light and CO_2 (Chapin *et al.*, 1987). Responses to simultaneous abiotic stresses can be influenced by ploidy level (Deng *et al.*, 2012), and by the effects of GS on the phenotype (e.g. cell size (Knight & Beaulieu, 2008)). We thus investigate how GS and ploidy influence a species' luminosity and soil moisture (water availability) niche attributes, which are inferred

from Ellenberg's indicator values for light and water. Negative correlations between water availability and GS might arise due to larger stomata size being more susceptible to water limitation and loss (Veselý *et al.*, 2012; Carta & Peruzzi, 2016). Light may have a negative association with GS where larger genomes are more susceptible to, or less able to repair, UV damage. Absorbance of ultraviolet B (UV-B) radiation can lead to mutations (e.g. pyrimidine dimers), and inactivation of photosystem II, and disruptions to cellular metabolism, transcription, and DNA replication (Jansen *et al.*, 1998). Genome size and N are hypothesised to show a positive association. If larger GS are more costly to build and maintain, species with larger GS should show higher demands for nutrients, particularly those that are competitive grassland species (Šmarda *et al.*, 2013; Guignard *et al.*, 2016).

Materials and Methods

Data

A taxon's realised niche values for nitrogen (N), water (W), and light (L), are represented by Ellenberg's indicator values which were estimated from species distributions in Central Europe. These were revised (Hill *et al.*, 1999) to reflect species ecological distributions in the British Isles (GB), and are freely available (also in Hill *et al.*, 2004). Descriptions of each indicator value analysed here and scaling are given in Table 4.1.

The competiveness of a species is based on Grime's plant strategies (C -competitive, S-stress tolerant, R-ruderal) (Grime, 1974) and was attributed following Hodgson et al. (1999), who also revised these according to how species behaved in GB. Grime's competitor strategy (C-strategy) reflects a taxon's ability to compete for resources. Highly competitive plants (C-strategy = 1) typically require at least mildly fertile soil (e.g. *Chamerion angustifolium* (Onagraceae) with N = 5, *Solidago gigantea* and *S. canadensis* (Asteraceae) with N=6). Conversely, some widespread and ubiquitous species (e.g. *Stellaria_media* (Caryophyllaceae), *Matricaria_chamomilla* (Asteraceae), *Poa*

annua (Poaceae)), and woodland species (e.g. *Allium ursinum* (Amaryllidaceae), *Arum maculatum* (Araceae) are designated as non-competitors (C-strategy = 0) and have high N-attributes (N=7).

Genome size and ploidy level data were obtained from the Royal Botanic Gardens Kew C-values database (Bennett & Leitch, 2012). We used the prime holoploid 1C-value, which is the amount of DNA in an unreplicated gametic nucleus in picograms (pg) (1 pg = 978 megabase pairs). Where GS was reported for more than one ploidy level within the same species, we viewed published chromosome counts (Rice *et al.*, 2015) and included the ploidy level that was most likely to represent species in GB, or if unavailable, species in Central Europe. The complete dataset comprises 462 taxa from 267 genera, distributed among 76 families and 33 orders (Table S4.1).

Statistical analyses

Analyses were carried out in R 3.13 (R Development Core Team, 2012). All analyses take into account phylogenetic correlation between taxa. We pruned the DAPHNE phylogeny (Durka & Michalski, 2012) using the R package "ape" (Paradis, 2004) to match species in our dataset. Where applicable and appropriate, tip labels were changed to those of a closely related species existing in the data available in Hill et al. (1999 and 2004). The presence of phylogenetic signal in C-value, competitor strategy (C-strategy), and species' attributes of nitrogen (N), light (L), and water (W) was estimated using Pagel's lambda and Blomberg's K-statistic with the *phytools* package (Revell, 2012), under 10,000 randomizations.

Essentially for visualization purposes, we first obtained the mean C-value for each level of each indicator value using phylogenetic least squares (PGLS) regressions, which were fitted assuming a Brownian motion model of evolution (packages "ape" and "nlme" (Paradis, 2004; Pinheiro *et al.*, 2013)), on log-transformed 1C-values, which were back-

transformed. We used weighting (i.e. 0, 1) to maintain the same phylogenetic structure for all analyses. We then fitted phylogenetic linear mixed effect models (PGLMM) using

Table 4. 1 Description of the indicator values (Hill *et al.*, 2004) used by Ellenberg *et al.* (1992) to classify plants according to where a species is usually found together with a description of other traits analysed.

	Description
Nitrogen	Represents soil fertility. Ranges from extremely infertile habitats (N = 1) to fertiliser-enriched sites (N = 9). An example of a habitat with N = 9 are cattle resting places.
Light	Values range from 3 to 9 for taxa in the British Isles. L = 3 is a shade plant, found in habitats with < 30% light; 5= semi-shade; 7= preference for light but may occur in partial shade; 9= full sun.
Water	Water availability / soil moisture. Ranges from soils that remain arid for periods of time (W = 1) to submerged taxa (W = 12). A value of W = 3 indicates preference for dry soils; 5 = moist sites; 7 = constantly damp soils; 9 = water-saturated soils; 10 = shallow water.
Competition	A species' C-strategy, from Grime's (1977) C-S-R functional types. Estimates competitive ability of a species. Most species are attributed a combination of strategies i.e. competitor, stress tolerator, and ruderal. C-strategy values in our data are: 0, 0.1667, 0.25, 0.3333, 0.4167, 0.5, 0.6667, 0.75, 1, where 1 = most competitive.
1C-value	Holoploid genome size. The three largest C-values in our dataset belong to species from the Liliales, e.g. *Paris quadrifolia* (Melanthiaceae). The smallest C-value (0.15 pg) is shared by three species: *Epilobium palustre* (Onagraceae), *Hypericum hirsutum* (Hypericaceae), and *Sedum album* (Crassulaceae).
Ploidy level	Ranges from 2x to 16x. Only two taxa are 16-ploid: *Ranunculus lingua* (Ranunculaceae) (1C = 21.1 pg), and *Cerastium fontanum* (Caryophyllaceae) (1C = 2.93pg); one taxon is decaploid: *Trifolium medium* (Fabales) (1C= 2.93 pg). Octoploids are represented by 10 species from 9 families.

the *MCMCglmm* function from the R package of the same name (Hadfield, 2010). We collapsed Ellenberg's indicator values, which are ordinal variables, into continuous variables. One reason for this is there is as yet no method in R that allows the implementation of an ordered multinomial logistic model for more than two levels. The second reason is that Ellenberg's values represent niche attributes occurring on a continuous scale in nature (e.g. along a gradient of complete shade to full sun for L).

We tested whether GS was a predictor of N, L, W, and C attributes in 462 species by fitting univariate PGLMMs, which were run with 2 million iterations each, and sampled every 200 intervals, including a burn in of 20,000. Species was treated as a random effect with the phylogeny as the covariance. We used weak priors where V= 1 and nu= 0.002. Model convergence was verified with trace plots, autocorrelation statistics, and Heidelberger and Welch's convergence diagnostics (Plummer *et al.*, 2010).

After univariate regressions, we used principal component analyses (PCA) to test how a species' realised N niche was influenced by GS, C-strategy, and the other niche parameters of light and water availability. We performed phylogenetic PCA (Revell, 2009) with a Brownian motion correlation structure representing the phylogeny and standardized variables. The first three principal components were then regressed on N in a PGLMM as described above.

We were also interested in the effects of polyploidy, and thus had to prune our data to only include taxa where ploidy levels were known (n= 356). Again for visualization purposes, we partitioned this dataset into diploid taxa and polyploid taxa, and pruned our phylogenetic tree to make one tree with diploid taxa only and one with polyploid taxa only. We estimated the mean 1C-value with PGLS for each level of each indicator value, as described above. We then fit a multivariate (i.e. multiple response) PGLMM with *MCMCglmm*, testing the effects of GS, competition, ploidy level, and their interactions, on a taxon's realized N, L, and W niche parameters. Genome size was log-transformed and we specified the "~us(trait)" variance structure to allow different variances across traits for N, L, and W and for covariances to exist between them. The intercept was removed to facilitate interpretation (Hadfield, 2010). Priors were specified with nu = 1.002 and a 3x3 covariance matrix and the model was run with four million iterations and burn in of 40,000.

Results

Phylogenetic signal was weakest for the C-strategy, N- and L-niche parameters (K = 0.180, 0.164, 0.172, lambda = 0.536, 0.458, 0.432 respectively). It is relatively high in W (K= 0.521, lambda = 0.906) and GS (K= 0.617, lambda = 0.968), indicating that closely related species tend to be more similar to each other in GS and in the realized niche parameter for water. The p-values of the phylogenetic signals were all significant (p= 0.0001 for K, p < 0.0001 for lambda).

A trend towards an increase in N with larger GS can be seen in Figure 1a (see also Table S4.2 for summary statistics of each trait and indicator value). Conversely, light and GS show a negative association (Fig. 4.1). The univariate regressions show an effect of a higher N value with larger GS (b = 0.04, pMCMC= 0.0402), and a decreasing effect of GS on L (b = -0.04, pMCMC = 0.008); note only L remains significant after a Bonferroni correction. No direct associations between GS and W (b = -0.007, pMCMC= 0.772), or between GS and C-strategy (b= -0.0003, pMCMC= 9.917) can be seen in the univariate regressions (see also Fig 1c, d).

Patterns of variation among GS, competition, L, and W were extracted in ordination space with a PPCA (Fig. 4.2). The first principal component (PC) is mainly represented by competition and W, accounting for 32.7 % of variance (Table 4.2a, b). Light is the main factor in PC2 and C-value accounts for most of the variation in PC3. Genome size and W point towards the same direction as competition on the PC1 axis, whereas light is negatively associated with GS (Fig 4.2). Regression of the N indicator value onto the first three PCs shows PC1 (predominantly competition and W) and PC3 (C-value) having the most significant effects on a species' N value (Table 4.2c).

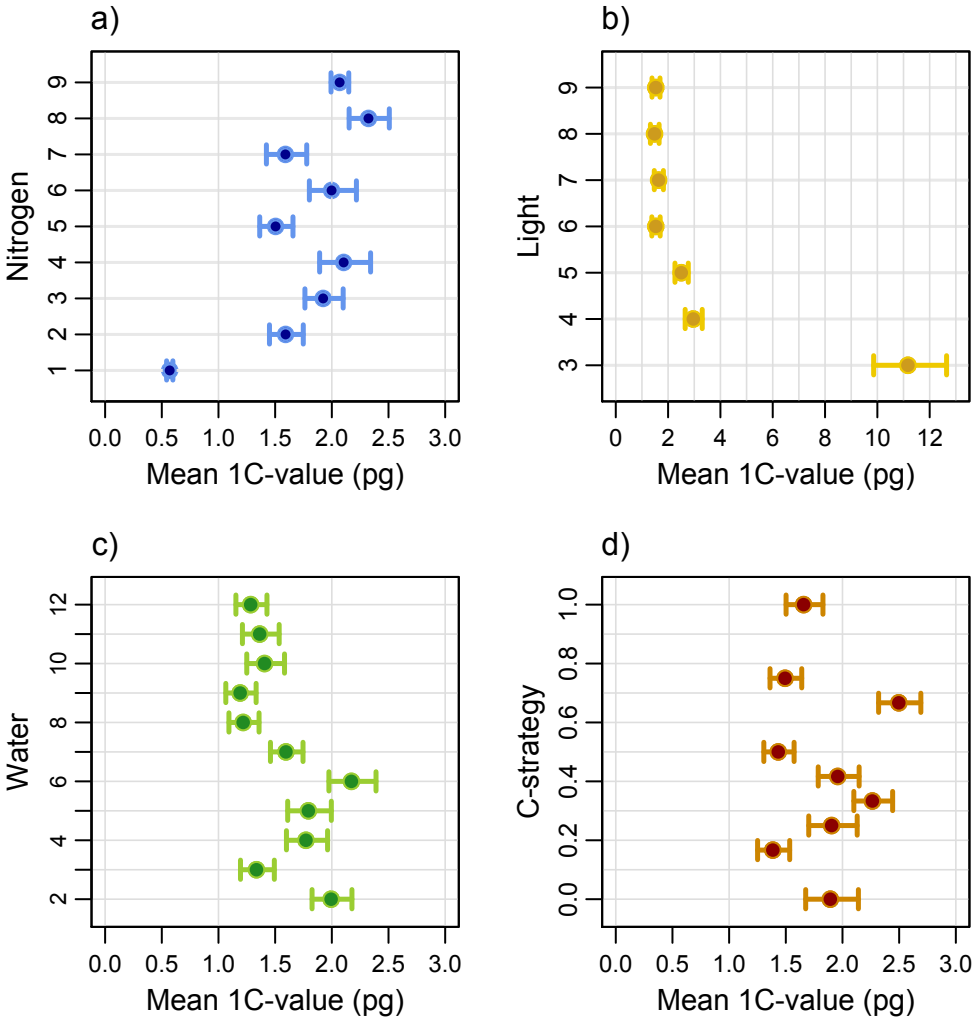

Figure 4. 1 Mean 1C-values for each level of indicator values N, L, W, and C-strategy. These were estimated with phylogenetic least squares regression (PGLS) where GS was log-transformed. Shown here are the back-transformed values and error bars with the 95% confidence interval.

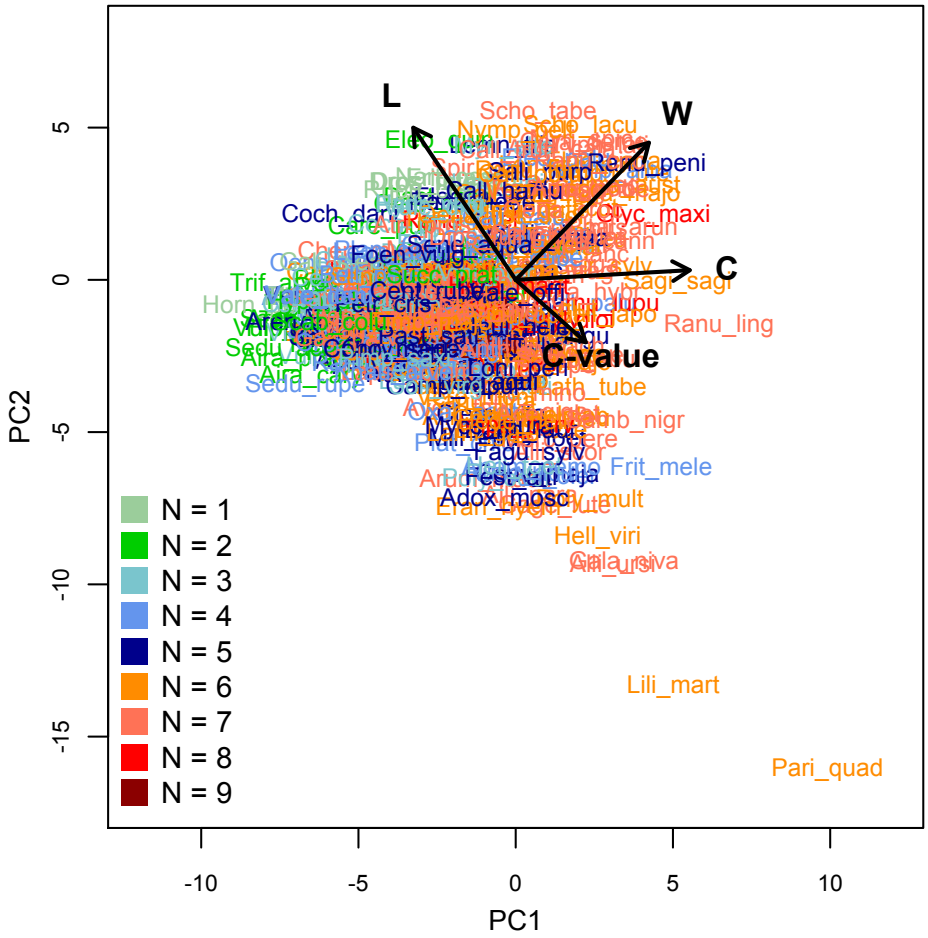

Figure 4. 2 Phylogenetic principal component analysis (PPCA) biplot showing how GS, a species' competitiveness (C, Grime's C- strategy), and indicator values for water (W) and light (L) map onto a species N attribute. Plots of principal components (PC) 1 and PC3, and of PC2 and PC3 are provided in the Appendix (Fig. S4.1).

Table 4. 2 a) Variance explained by each phylogenetic principal component; **b**) loadings; and **c**) PGLMM regression output testing the effect the first three principal components on species N scores. Sample sizes = 462.

a) Importance of components	PC1	PC2	PC3	PC4
Standard deviation	1.144	1.005	0.986	0.841
Proportion of Variance	0.327	0.253	0.243	0.177
Cumulative Proportion	0.327	0.580	0.823	1.000

b) Loadings	PC1	PC2	PC3	PC4
Light	-0.465	0.714	-0.384	0.356
Water	0.605	0.643	0.008	-0.469
C-value	0.318	-0.293	-0.899	-0.070
Competition	0.791	0.045	0.130	0.597

Table 4.2 continued

c) *MCMCglmm*	Posterior mean	Lower & upper credible intervals	Effective sample size	pMCMC
Intercept	5.32	4.33, 6.31	8909	0.0001
PC1 (C + W)	**0.37**	**0.31, 0.42**	**9900**	**0.0001**
PC2 (L + W)	**-0.08**	**-0.14, -0.01**	**9900**	**0.0200**
PC3 (GS)	**0.09**	**0.03, 0.15**	**8761**	**0.0040**
G-structure (random effect)	2.018	0.97, 3.20	9900	na
R-structure (residuals)	1.480	1.15, 1.81	9900	na

Partitioning the data into diploid and polyploid species shows that polyploids generally have larger GS than diploids within most attributes and levels (Fig. 4.3). The N attribute of polyploids tends to increase with GS, and low light (L attribute) is associated with large GS, in both diploids and polyploids. There are no visible direct trends between moisture (W) and C-strategy (Fig. 4.3). A multivariate PGLMM shows significant three-way interactions between GS, ploidy, and competition (C-strategy), and significant two-way interactions between GS and competition on N and W (Fig. 4.4, Table 4.3). Results are similar for N and W, however C-value and the interaction between C-value and ploidy show significant effects on N but not on a taxon's W value. Light and GS are negatively associated, while ploidy and competition play no role on a taxon's niche attribute for light. Ploidy has an effect only when it is coupled with GS, or with C-strategy. Phylogeny explains a large part of the variance in W (Table 4.3b), as is also shown by the phylogenetic signal metrics given above, but also in L. The indicator value for N shows the highest residual variance (Table 4.3c).

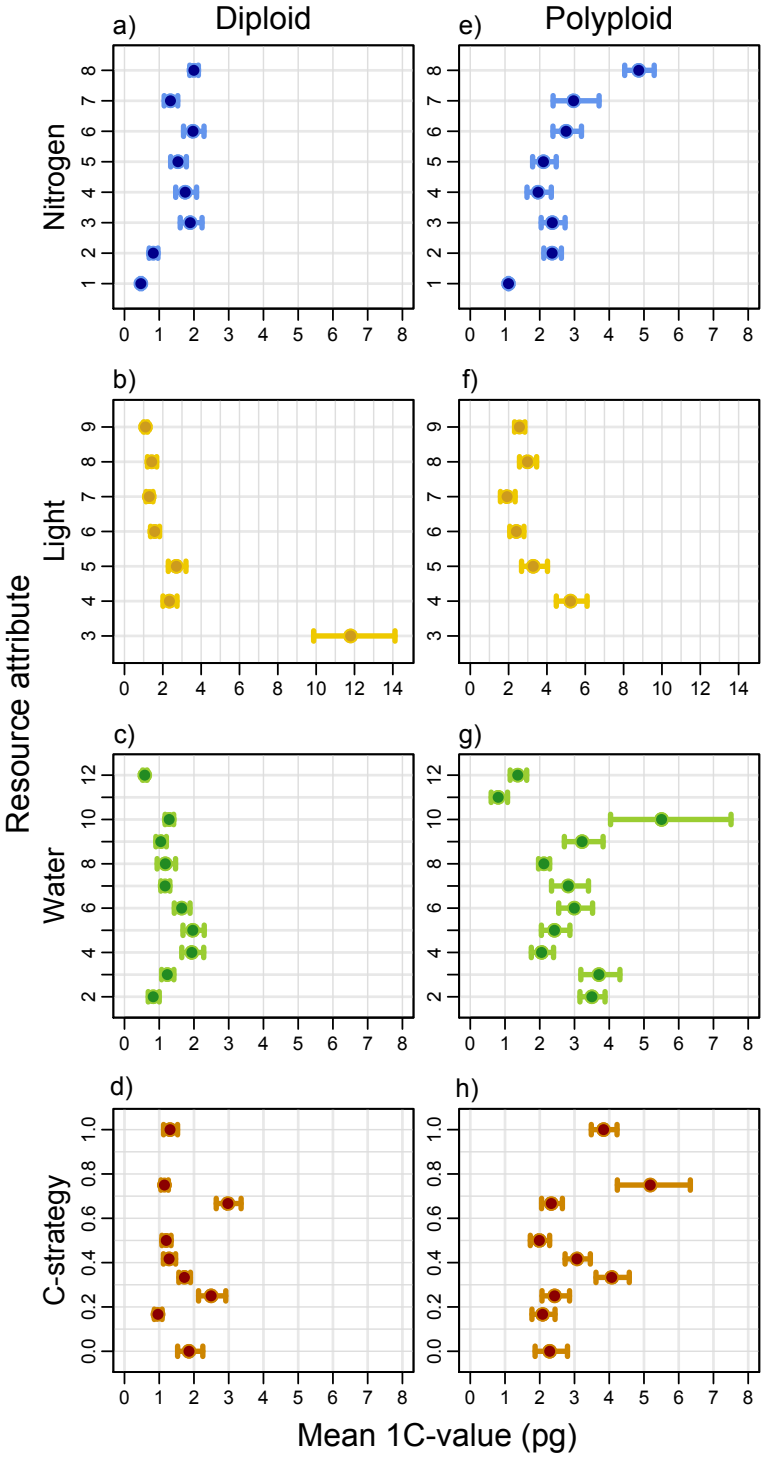

Figure 4. 3 PGLS mean 1C-values for each level of each indicator value and C-strategy in diploids (2n) (**a-d**) and polyploids (**e-h**) (n=214, 142 respectively). Only two species in our data were scored with N= 9: *Artemisia absinthium* (Asteraceae), a diploid with 1C= 1.53 pg; and *Rumex obtusifolius* (Polygonaceae), a tetraploid with 1C= 3.65 pg. *Callitriche obtusangula* is the sole 2n species with a W-value of 11; it has a 1C-value of 1.83 pg. *Mercurialis perennis*, is the only polyploid (8x) with a very low light value; it has a 1C = 2.35 pg). Error bars show 95% confidence intervals.

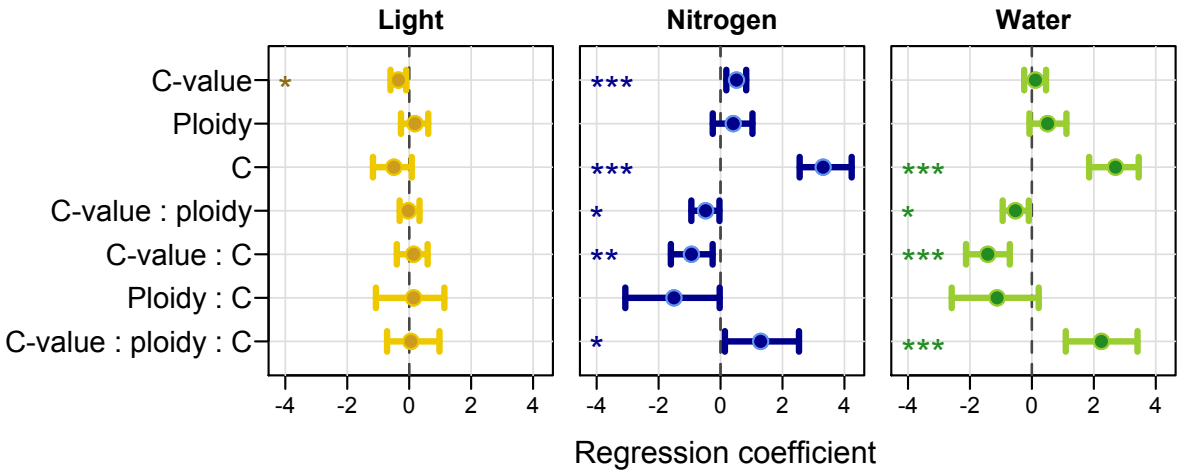

Figure 4. 4 Effects of genome size (1C-value), ploidy, and C (C-strategy or competition) on the indicator values (i.e. realized niche) for light, nitrogen, and water (soil moisture), estimated with a multivariate phylogenetic mixed effect model (PGLMM). C-value was log-transformed. The error bars show the 95% confidence intervals. Table 4.3 shows the coefficients and G- and R-structure. P-value < 0.05 *, p < 0.01 **, p < 0.001***

Discussion

These results show that GS and ploidy level influence the N value of a species, i.e. its realised abiotic niche in terms of soil fertility. This association is shown to be both direct (i.e. species occupying niches characterised by high N values have higher GS and ploidy levels than those occupying N-poor niches) and indirect via interactions with a species' competitive ability (C-strategy) (i.e. those species occupying high N sites tend to more competitive). A species' N value also occurs in association with other niche attributes (i.e. light and water), but especially water. This coupling of N and water may be linked via the effects of GS on cell size (larger GS tend to have large cells) (Knight & Beaulieu, 2008; Šímová & Herben, 2012) and how this, in turn, can impact photosynthetic and water use efficiency. Approximately 75% of leaf nitrogen is invested in chloroplasts, which is then primarily invested in photosynthesis (Chapin *et al.*, 1987). Larger genomes, which would require more N investment, are positively correlated with stomata size

Table 4. 3 Summary of multivariate (multiple response) PGLMM output, performed with the *MCMCglmm* function, testing the effects of GS (C-value), ploidy, and competition on indicator values N, W, and L for 356 taxa. Note that C-value was log transformed. **a**) Coefficients of for each dependent and each fixed effect and their interactions; **b**) the G-structure shows the phylogenetic (co)variances; **c**) the R-structure shows the residual (co)variances. Credible intervals show upper and lower 95% intervals. Significant parameters are highlighted in bold ($\alpha < 0.05$).

a)

Dependent variable	Fixed effects	Posterior mean	Credible intervals	Effective sample size	pMCMC
	C-value	**-0.350**	**-0.60, -0.10**	**1000**	**0.01**
	Ploidy	0.185	-0.27, 0.61	1000	0.418
	Competition	-0.493	-1.17, 0.08	1000	0.114
L	C-value : ploidy	-0.031	-0.31, 0.33	1000	0.842
	C-value : competition	0.144	-0.40, 0.60	1000	0.538
	Ploidy : competition	0.143	-1.07, 1.14	1000	0.792
	C-value : ploidy : competition	0.056	-0.71, 0.98	807.5	0.906
	C-value	**0.516**	**0.19, 0.83**	**1000**	**<0.001**
	Ploidy	0.410	-0.25, 1.03	1000	0.2
	Competition	**3.313**	**2.55, 4.23**	**1001.3**	**<0.001**
N	**C-value : ploidy**	**-0.481**	**-0.94, -0.04**	**1146.6**	**0.042**
	C-value : competition	**-0.940**	**-1.60, -0.26**	**834.9**	**0.006**
	Ploidy : competition	-1.504	-3.07, -0.03	1000	0.056
	C-value : ploidy : competition	**1.296**	**0.14, 2.53**	**1000**	**0.044**
	C-value	0.113	-0.25, 0.46	1718	0.528
	Ploidy	0.507	-0.08, 1.11	1000	0.112
	competition	**2.700**	**1.85, 3.45**	**1000**	**<0.001**
W	**C-value : ploidy**	**-0.531**	**-0.94, -0.10**	**1000**	**0.01**
	C-value : competition	**-1.425**	**-2.13, -0.71**	**1000**	**<0.001**
	Ploidy : competition	-1.124	-2.59, 0.22	1000	0.126
	C-value : ploidy : competition	**2.244**	**1.09, 3.41**	**1000**	**<0.001**

Table 4.3 continued

b)

Dependent variable	G-structure	Posterior mean	Credible intervals	Eff. sample size
L	N	2.073	1.30, 2.97	389.3
	L	4.946	3.90, 5.94	550.4
	W	3.360	2.32, 4.38	488.7
N	N	3.259	2.00, 4.60	173.8
	L	2.073	1.30, 2.97	389.3
	W	2.155	0.96, 3.25	215.5
W	N	2.155	0.96, 3.25	215.5
	L	3.360	2.32, 4.38	488.7
	W	6.510	4.46, 8.26	330.6

c)

Dependent variable	R-structure	Posterior mean	Credible intervals	Eff. sample size
L	N	-0.563	-0.76, -0.35	330.9
	L	0.552	0.37, 0.72	345.7
	W	-0.347	-0.54, -0.17	365.5
N	N	1.606	1.22, 2.07	255.2
	L	-0.563	-0.76, -0.35	330.9
	W	0.050	-0.23, 0.35	302
W	N	0.050	-0.23, 0.35	302
	L	-0.347	-0.54, -0.17	365.5
	W	1.138	0.79, 1.55	338.2

(Beaulieu *et al.*, 2008), which are much more susceptible to water loss (Hetherington & Woodward, 2003; Drake *et al.*, 2013). Low water availability is linked with low leaf nitrogen content, and to diminishing returns in photosynthesis when water and N availability are mismatched (Chapin *et al.*, 1987). Species with very large GS are associated with more shaded niches. Plants in open areas are exposed not only to higher, but also fluctuating levels of UV-B radiation. Enzymatic and non-enzymatic systems are involved in defences from harmful radiation, which may be constantly adjusted with

changing UVB levels (Jansen *et al.*, 1998). Such complex processes are likely to be less efficient and more costly in taxa with very large GS. Lower light may also be correlated with more humid areas, although in our data the correlation between a species N and L values is very small (rho= -0.0211, p=0.65). Taxa with a larger GS may be also be experiencing competition from faster growing species for light.

The associations reported above are noisy by nature, as they are based on relatively very simple and coarse ecological values, and would also be influenced by climatic variables (e.g. temperature), atmospheric variables (e.g. CO_2), geography (latitude and altitude), biotic interactions, and finally, stochastic processes (Chase & Myers, 2011). The interface between plant species and habitat variables are shaped by complex and intricate dynamics (Craine & Dybzinski, 2013; Farrior *et al.*, 2013) which are of a multidisciplinary nature (Chapin *et al.*, 1987). Yet despite a multitude of potential confounding effects, the data show highly significant interactions between the realised niche of a taxon with GS and ploidy. These two genomic parameters, and cryptic cytotype diversity (variation in chromosome numbers and ploidy levels within a species) are known to also influence the range of a species' climatic niche (Thompson *et al.*, 2014; Sonnleitner *et al.*, 2016). There is increasing evidence that limiting nutrient availability may act as a selection pressure against larger genomes in plants (Šmarda *et al.*, 2013; Kang *et al.*, 2015; Guignard *et al.*, 2016) and that taxa with larger genomes are more demanding of macronutrients for growth processes (e.g. aquatic micro-organisms (Hessen *et al.*, 2008; Jeyasingh *et al.*, 2015). Structural, physiological, and biomechanical adaptations to abiotic stress (Lynch & Clair, 2004; Osakabe *et al.*, 2014) will provide confounding factors and prevent tight ecological correlations between GS and realized niche parameters.

One factor which is likely to contribute to the selection against plants with large genomes under limited N environments is the costs of the elements (N and P) needed for the construction of nucleic acids and histones. While the coding content of angiosperm

genomes (i.e. the proportion of the genome comprising the gene space) is likely to vary by only a few fold between large and small genomes, it is differences in the amount of non-coding, often highly repetitive DNA sequences which contribute to GS diversity, with this fraction comprising an increasingly significant proportion of the genome in species with larger GS (Grover & Wendel, 2010; Slotkin *et al.*, 2012). Despite their lack of apparent function, much of it can be transcribed, indeed transcription of repeats is at the heart of epigenetic silencing mechanisms (Matzke & Mosher, 2014). Thus with increased GS there is likely to be increased RNA transcription, further adding to the burden of N-demanding nucleic acids. Approximately 30% of the transcriptome can change in response to stress brought on by drought and cold (Kreps *et al.*, 2002). Smaller GS may allow more efficient responses to environmental stress, such as the up-regulation of microRNAs under P-starvation (reviewed in Sunkar *et al.*, 2007) and more rapid nucleic acid replication.

It is becoming increasingly important to understand the factors driving our planet's primary productivity, and how plants, at the individual, species, and community level, respond to environmental change (Craine *et al.*, 2012). Anthropogenic activities, by affecting the climate and the environment, are having direct impacts on a plant's access to resources (nutrients, light, water) and indirect impacts (e.g. via changes in soil pH, temperature, atmospheric pollution). Increased N inputs from fossil fuels and the Haber Bosch reaction for fertilisation has altered nitrogen to phosphate ratios (Peñuelas *et al.*, 2012). As GS and polyploidy are emerging as important genomic traits linked to species biomass production, and distribution across different environments and ecosystems, their incorporation into models (e.g. Shipley *et al.*, 2006; Laliberte *et al.*, 2012) seeking to understand how species and communities respond to external factors and environmental stresses is essential to improve understanding and reliability of the predictions.

Chapter 5 General Discussion

Summary

Data presented in this thesis show support for the hypothesis that larger genomes are more costly in terms of N and P requirements, and are selected against, especially in habitats with low nutrient availability. The Park Grass experiment shows that species growing on the site with large genome sizes increase in above-ground biomass on the high nutrient plots in comparison to: 1) the amount of biomass they produced on low nutrient plots (no fertilizer, N-only, and P-only) plots; and 2) in comparison to taxa with smaller GS. Polyploid species are also under limitation by N and P; this can be seen by the increased polyploid biomass on plots where these two elements are added as fertilizer (e.g. from a mean of 60.9 ± 11.3% on P-only plots to mean 74.7 ± 10.5 % on N+P plots). Species indicator values, which represent the realized niche of a species, show a positive association between GS and the N-niche attribute, which corresponds to soil fertility. The Nash's Field experiment at Silwood Park showed that mean plant GS are also lower in plant communities subject to rabbit grazing pressure. This may be due to a lower tolerance to damaged parts and the associated cost of regrowth, and/or preferential grazing by rabbits in species with large GS.

As far as I know, work in this thesis presents for the first time the effects of GS, polyploidy, N and P, herbivory and their interactions on plant biomass and abundances, These findings set the scene for many exciting avenues for further research, especially by taking these findings from grassland experiments to natural ecosystems. The outcome is likely to show that GS and ploidy levels are important genomic variables to consider when asking questions about the effects of macronutrient limitation on GS and polyploidy in an ecological context, on the growth rate and stoichiometry of a plant, and on the stoichiometry of its genome.

Interactions between genome size and ploidy level

This thesis reveals that there are not only ecological effects of GS and polyploidy, but also additional effects caused through their interactions. In the Park Grass experiment it was shown that diploid taxa with small GS produce more biomass than diploid taxa with large GS. Conversely, being a polyploid with a large GS is the most advantageous combination in a competitive grassland setting where nutrients are readily available. Polyploid taxa generally produce more above-ground biomass than diploid taxa (Fig. 5.1a), but this varies with GS and nutrient availability. Within a plant community context, these two genomic traits may be playing different roles, where GS is a constraint in terms of nutrient availability and polyploidy is an advantage in terms of competitive ability, arising through advantages of, for example, fixed heterozygosity. It seems also apparent at Park Grass that polyploid taxa are producing most of the above-ground biomass, but diploid taxa are contributing the most to species diversity. Further sources of variation, which likely influence a plant's nutrient uptake and competitive ability, include soil pH, type of nitrogen fertilizer applied, soil microbe community, and grazing pressure.

Plant community studies typically focus on traits that are plastic and quantifiable (e.g. specific leaf area, biomass, N uptake) or categorical (e.g. woodiness, grass vs forb) to measure species ecological preferences and responses to environmental conditions. Violle et al. (2007) defined a functional trait as that which affects growth, reproduction, and survival and thus has an indirect impact on fitness. The correlations between GS and cell size, seed size, cell cycle time, and photosynthesis rates makes GS a prime candidate for consideration as a functional trait. The effects of polyploidy, including fixed heterozygosity, gene redundancy, and self-compatability may influence a taxon's growth and survival and fitness, at least in the short term, before genome diploidizing processes become apparent. Unlike most other plant traits, which typically measure plant morphology, phenology or physiological

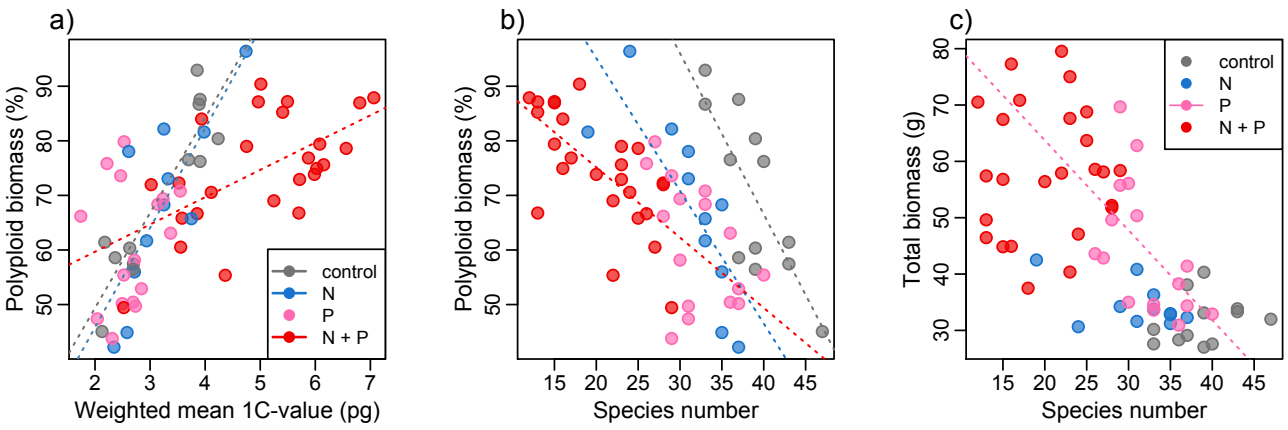

Figure 5. 1 Correlations between GS, polyploid abundance, species numbers, and total biomass of plots at the Park Grass experiment. **a**) Correlations between polyploid abundance and mean genome size varied between different plots which differ in their nutrient treatment, where the strongest correlations were on control plots (no fertilizer) (Spearman's correlation coefficient (rho) =0.736, p =0.0064) and on plots with N only (0.738, p= 0.0095). For plots receiving high levels of nutrients, polypoid abundance and GS were also relatively highly correlated (0.56, p= 0.0042), however on plots which were fertilized only with P, the relationship between the two traits was very weak (0.182, p= 0.4979). **b**) Number of species is negatively correlated with polyploid abundance: control plots, rho =-0.794, p= 0.0089); N plots, rho = -0.794, p< 0.0001; N+P, rho= -0.676, p= 0.0002. There was a lack of significant correlation on P-only plots (-0.476, p= 0.0745). **c**) The number of species is only correlated with total biomass on plots with P fertilizer. Control: rho = 0.276, p= 0.385; N: rho =-0.327, p= 0.326; P: rho=-0.635, p= 0.0082; N+P: rho=0.088, p= 0.6759). Number of plots analysed for each treatment = 12, 11, 16, and 25 for control, N, P and N+P respectively.

processes and which show both intra and interspecific variation, GS and ploidy level are plastic only at the community level (unless different cytotypes are established in sympatry). Genome size is a trait that is continuous yet constant (or nearly so) within a species or cytotype, whereas ploidy level can be classified as a categorical trait and defined as a binary character (for simplicity) when the exact polyploid level is unknown. Unlike most categorical traits, for example, "forbs" and "grasses", GS and ploidy are evolutionarily labile traits and transcend phylogenetic groupings. The data presented in this thesis strongly suggest that the consideration of GS and ploidy level as plant

functional traits will provide new ecological insights, particularly where research is focused on determining which traits underlie plant abundance (Laliberte *et al.*, 2012), biomass and species richness (Reich *et al.*, 2012), functional diversity (Dìaz & Cabido, 2001), invasiveness (Matzek, 2012; Pyšek *et al.*, 2012), plant community structure (Avolio *et al.*, 2014), and realized niche (Kraft *et al.*, 2015). As an example, under various nutrient treatments, species number is highly correlated with polyploid abundance (Fig. 5.1b), more so than with total biomass (Fig. 5.1c). Understanding the associations between species richness and standing biomass would be enhanced by integrating ploidy level and GS, as these influence plant growth and distributions, and correlate even with species numbers. Similarly, studies on the ecology and characteristics of polyploid taxa may gain additional insights by integrating GS.

Thresholds in GS and polyploidy

The data analysed here show that a larger GS is advantageous to polypoid taxa when nutrients are in plentiful supply, perhaps because biomass production is easier to achieve with larger cells, which may promote the growth of larger leaves, stems, or roots, thus occupying more ground space and shading out rivals. A GS threshold of 1C > 25 pg was suggested by Bennett (1972) to limit a taxon's ability to undergo sufficiently fast growth to adopt an annual life strategy and thus species above this GS are obligate perennials. Potentially the costs associated with possessing a large GS, for example, nutrient costs (especially N and P), cell structural costs (building larger cell sizes to accommodate a large genome, Beaulieu et. al. 2008) and mechanical costs (slower rates of cell division and slower growth rates) may vary with environmental conditions (including both biotic and abiotic factors) and impact such a precisely-defined threshold. Establishing what any threshold might be, would then be context-dependent. Similarly, there is likely to be a threshold on polyploidy where the number of chromosomes and ploidy level become so high that any competitive advantage incurred by polyploidy, is then offset by the

associated costs. There may also be a minimum GS threshold, where GS has no influence (or too small an influence to be detectable) on the growth of a species. The next step towards understanding constraints imposed by GS are growth experiments, where nutrient gradients, on fast-growing, competitive taxa with a range of GSs are examined in search of maximum and minimum GS thresholds.

Angiosperm genome size and N and P stoichiometry

Knowledge is lacking about how GS or ploidy influence the elemental composition (i.e. stoichiometry) of angiosperms, and we also know little about how RNA and DNA content of a cell/organism, both of which are rich in N and P, scale with GS and growth (Hessen *et al.*, 2010). Surprisingly little is known about how the growth rate hypothesis (GRH) (Elser *et al.*, 1996) applies to vascular plants, and even less to angiosperms. The GRH, or RNA-protein model, proposes that growth is limited by protein synthesis rates, which require ribosomal RNA and which are costly in P (Vrede *et al.*, 2004). In heterotrophs, fast-growing zooplankton contain up to 1·5% P and 10% RNA of their dry weight, while slow-growing zooplankton contain 0·6% P and 2% RNA (Makino *et al.*, 2003). In autotrophs N plays a more prominent role as it is invested in ribulose bisphosphate carboxylase (Rubisco), which contributes 20-30% of leaf N (Feller *et al.*, 2008), and is the most abundant protein on land (Raven, 2013).

Few studies directly address growth rate and N and P stoichiometry in vascular plants. Ågren (2008) proposes that plant C uptake is proportional to the amount of N allocated in proteins, which in turn is proportional to the quantities of P allocated to ribosomes, and highlights different relationships between organism N and P and growth rate. In birch (*Betula pendula*) seedlings and tomato plants (*Solanum lycopersicum*) C:N ratios increased linearly with growth rate whereas C:P ratios increased quadratically (Ågren, 2004, 2008). Matzek & Vitousek (2009) determined N:P ratios and protein : RNA ratios between fast and slow-growing (pygmy) pines (Pinaceae), both in their natural habitat

and in greenhouse growth experiments. The measurements taken in the field were consistent with the GRH, where the pygmy pines had higher N:P ratios, higher protein : RNA ratios, and lower concentrations of N, P, proteins, and RNA than the fast-growing pines. Significant positive correlations between N and growth rate, and between N : P ratios and growth rate, have also been reported by Peng et al. (2011) in whole plant seedlings of seven woody eudicot species, but not in four herbaceous Asteraceae species (which included annual, biennial and perennial life cycles). The authors found an absence of significant association with P and growth rate. Reef et al. (2010) investigated RNA : DNA ratios in two mangrove species from two families (Verbenaceae and Rhizophoraceae). RNA : DNA ratios of the vascular cambium tissue ranged 8.7-fold (between 1.8 -17.5) and 8.8- fold (0.6 and 5.9) and showed a significant positive correlation with growth rate, and significant negative correlations with C:P and C:N ratios, consistent with the GRH.

In order to address the GRH in plants, I undertook experiments with *Arabidopsis thaliana* Ler to investigate relationships between C, N, P, RNA : DNA ratios, and plant growth. Diploid and tetraploid seeds were grown with high and low N and P (see Fig. 5.2 for more details). Although there were no differences between the two ploidy levels, the results do support the GRH (Fig. 5.3). RNA : DNA ratios were highest in plants grown on N+P substrate, were positively correlated with leaf N (Fig 5.3a), and with decreasing C : N ratios (Fig 5.3c). RNA : DNA ratios may also be correlated with leaf P (Fig. 5.3b), except in polyploid plants grown on P-only substrate (data on diploid plants is lacking), which have surprisingly low RNA abundance. This may be because N is needed to mobilise the P.

Whole plant stoichiometry is challenging to estimate due to the size and structure of plants. Variations in elemental ratios between stems, leaves, roots, and even between leaves within the same plant require enough replicates to obtain reliable estimates.

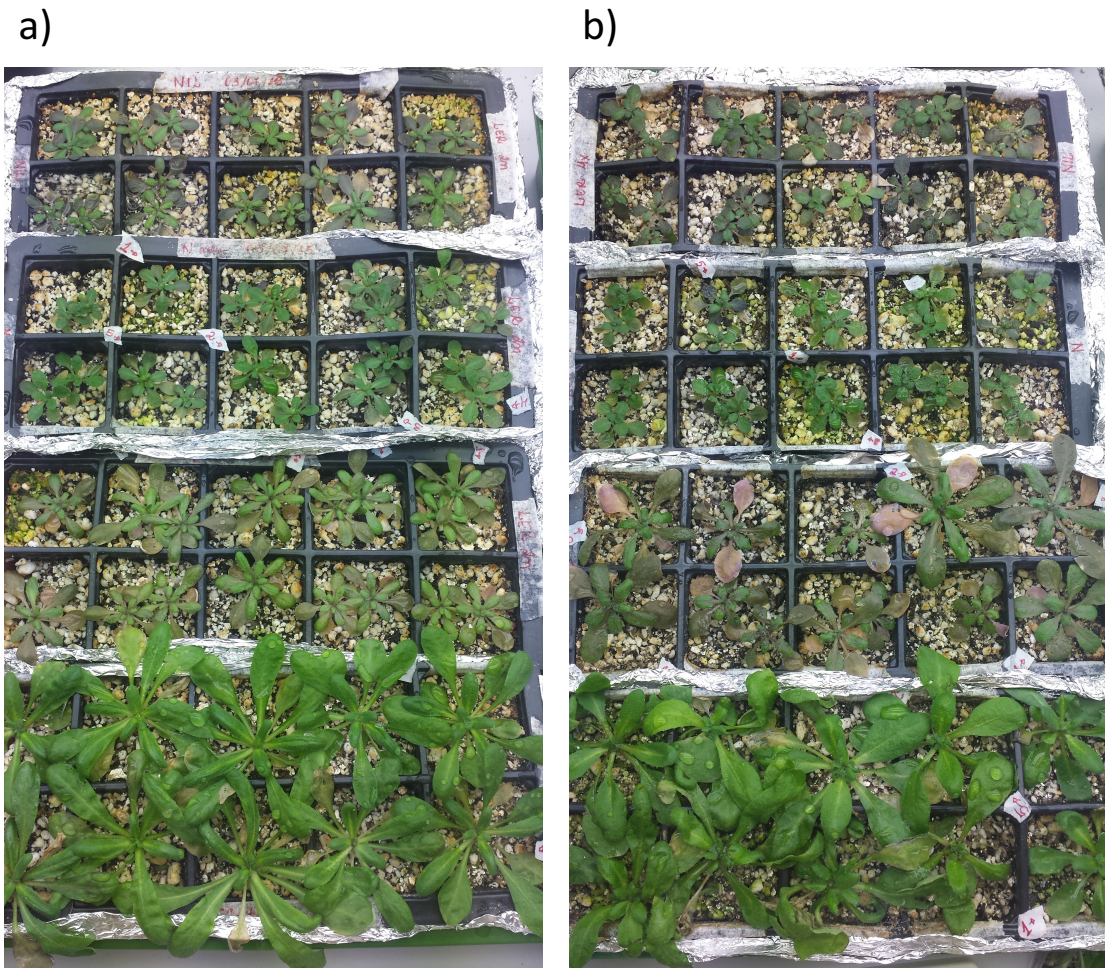

Figure 5. 2 *Arabidopsis thaliana* (background line Landsberg erecta (Ler)) growth experiments, with **a**) diploid plants; and **b**) synthetic autotetraploid plants. Very briefly, seeds were planted in four nutrient treatments with modified Murashige and Skoog (MS) (1962) nutrient mix: 1) control (no N, no P); 2) no N; 3) no P; and 4) N+P (i.e. normal MS). These treatments can be seen from top to bottom in the figure above, where two rows are allocated to each treatment (i.e. ten replicates). Growth substrate consisted of a 3:2:1:1 ratio of perlite, vermiculite, sand, and coir and a 4:1 ratio of substrate to nutrient solution. The substrate was autoclaved and given two weeks for nutrients to be absorbed, with intermittent mixing. Plants were watered with distilled water, and fortnightly with a weak nutrient solution (1/6th MS) and grown at a constant temperature (20°C) with 24-hour light regime. Leaf samples were collected before flowering and oven-dried for elemental analyses, and flash frozen for RNA : DNA ratios. Carbon and N were measured by combustion with mass spectrometry, and P was measured with a segmented flow analyser following persulfate digestion. RNA: DNA ratios were estimated with a Plant RNA/DNA Purification Kit (Cat. 24400) from Norgen Biotek.

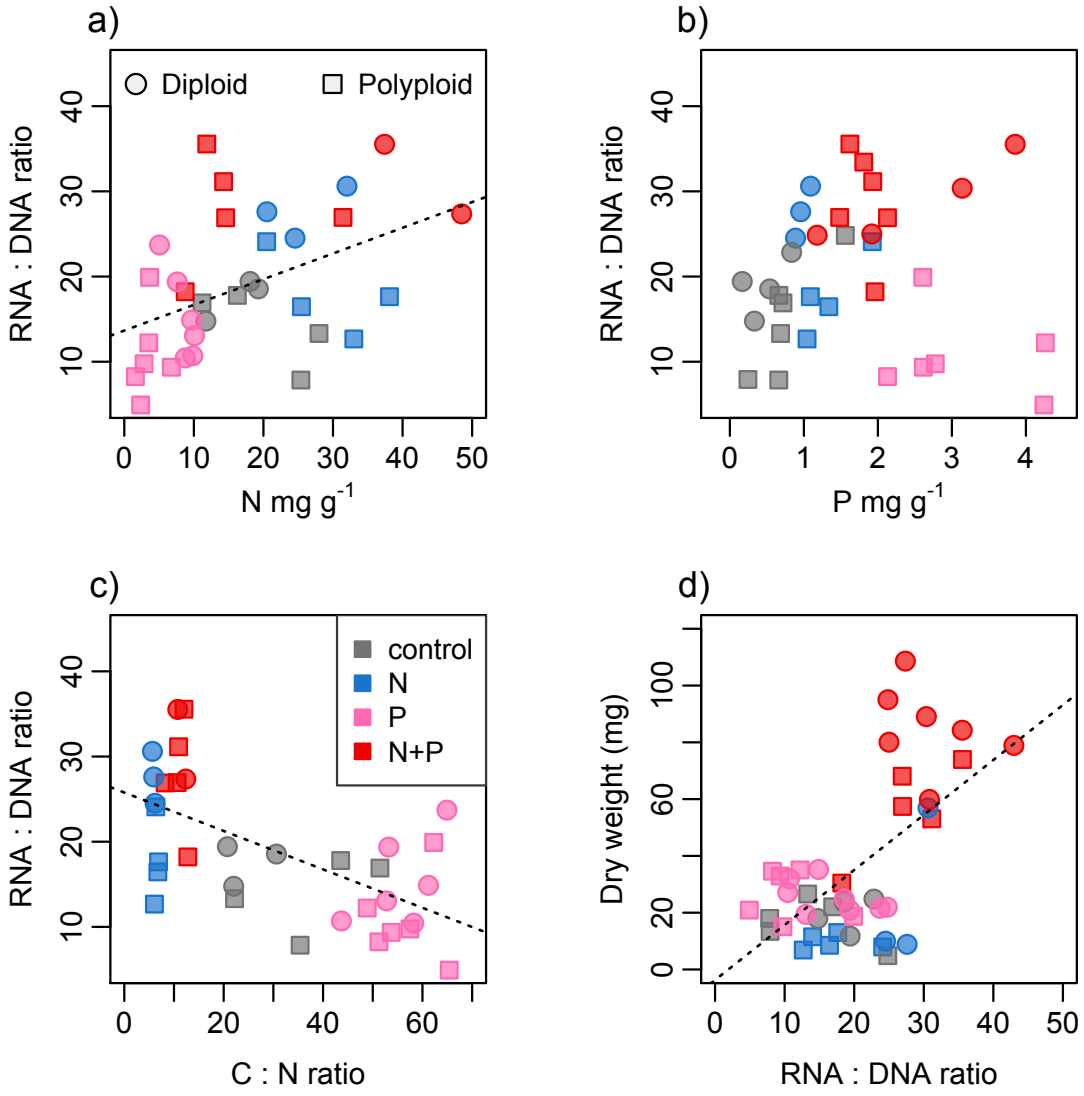

Figure 5. 3 Correlations between RNA:DNA ratios and elemental content in *Arabidopsis thaliana*, grown in four nutrient treatments. Circles represent diploid plants, squares represent tetraploid plants. A dotted line is shown when the correlation through all data points is significant. RNA : DNA ratios correlate significantly with **a**) leaf N dry weight (Pearson's coefficient (r)= 0.444, p= 0.0096); are not significant with **b**) leaf P dry weight (r = 0.029, p= 0.873); **c**) show a negative correlation with C:N ratios (r = -0.616, p=0.0001), and **d**) show a positive correlation with total plant dry weight (r= 0.612, p< 0.0001).

Another challenge is the accumulation of excess N and P, and finding the critical N : P ratio, where both elements are maintained at a level that is sufficient to allow growth yet is sufficiently limiting to prevent storage (Ågren, 2004). These are preliminary results, and growth experiments with fast and slow growing species (e.g. Grime's (1977) competitive, stress tolerant and ruderal adaptive plant strategies), and species with small and large GS, will provide further insights into the allocation of N and P and whether plants with diverse growth strategies, ploidy levels, and GS maintain homeostatic stoichiometry.

Stoichiometry in the genome

In a comparison between nine plant (including seven crop species) and nine animal genomes, N conservation was found in plant genomes but not in animal genomes (Elser *et al.*, 2006; Acquisti *et al.*, 2009). The analyses suggested that the nitrogen content in the proteome was highest in crop plants, lowest in undomesticated plants, and intermediate in nitrogen-fixing legumes and animals (Elser *et al.*, 2006; Acquisti *et al.*, 2009a). Though such results were interpreted as indicating a selective response to limitations in N, they need to be interpreted with caution, because they did not take phylogenetic relationships into account. A similar analysis, analysing the data within a phylogenetic framework should ideally be performed. Furthermore, fitness and transcriptome comparisons of plants grown on N-limited and N-unlimited substrate would also be informative. Another avenue for future research would be to compare genomes and transcriptomes of modern crops, with ancient landraces and undomesticated wild relatives; where N efficiency would be expected in the wild species, and perhaps early crop landraces, but not in modern crop cultivars.

How prevalent are the effects of GS in plant community ecosystems?

The combined influence of macronutrients, GS and polyploidy on plant biomass and distributions was shown at long-term natural experiments in Rengen (Šmarda *et al.*, 2013) and Rothamsted (Guignard *et al.*, 2016). However, this effect of increased GS with increased nutrients was not seen in plant communities growing on Nash's Field at Silwood Park. Potentially the differences reflect different community structures, intense herbivory at the Nash's Field site, or the young age of that experiment (25 years, and data analysed were means from five and eight years after the start). A future direction of research could test these hypotheses, by integrating the ideas in this thesis to the 40 plus grassland experiments set up around the world by the Nutrient Network (NutNet). In addition, it is possible to exploit freshwater ecosystems, which carry a rich diversity of macrophytes ranging in GS (e.g. *Myriophyllum* sp. (Haloragaceae) (1C ≤ 0.5pg) to *Sagitaria sagitofolia* (Alismataceae), 1C= 21.25 pg) and cytotypes (e.g. *Ranunculus* (Ranunculaceae), *Callitriche* Plantaginaceae)) (Rice *et al.*, 2015) that are amenable to field and experimental surveys, since semi-natural tanks (mesocosms) can be readily constructed. Freshwater systems vary in N and P concentrations, often due to anthropogenic inputs, and could thus provide a natural setting to test how plant abundances and distributions are influenced by GS, ploidy, and nutrient availability (Leitch *et al.*, 2014), as it has been shown with lake snails (Neiman *et al.*, 2013).

If GS and ploidy influence plant distributions and stoichiometry as we suspect, then it is highly likely that plant GS has ramifications through the food chain and to nutrient cycling, whether the plant is consumed fresh or as detritus. Environmental N and P influence both food quantity and quality, which have implications on the growth rates (Krist *et al.*, 2016) and food selection and behaviour of grazing animals (Ball *et al.*, 2000). At Nash's field sites, the data indicated opposite trends between GS and extent of herbivory between herbivore guilds, with lower mean GS in plant communities with rabbit grazing, and high mean GS in plots with mollusc grazing. At present, the driving

forces can only be hypothesized about and much more work is needed to fully understand these results. This work would involve acquiring more knowledge on the effects of different genomic parameters on plant elemental composition, on plant tolerance to damage by herbivory, and on animal preferences.

Conclusion

A plant's genome size is a consequence of many processes, and similar genome sizes in today's species will have been acquired via very dissimilar evolutionary histories. These include processes at the genomic level, including whole genome duplication but also insertions, deletions, and aneuploidy. These also include processes at the ecological level, where interactions between a plant's genome size, genotype and phenotype determines its ability to survive under biotic and abiotic stresses. All processes include some element of stochasticity, and to varying degrees and at multiple scales. Genome size and ploidy level are two of many parameters in the vast reticulated networks that comprise ecological dynamics, which include both stochasticity and selective forces. Nonetheless, it is clear that GS and ploidy do play a role in shaping plant community structure, by influencing angiosperm species' responses to different types of environmental stresses, such as macronutrient limitation, competition, and herbivore-induced pressure and environmental change.

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Appendix 1 Supporting information for Chapter 2

Supporting Figures

Supporting Tables

Methods S2.1 Phylogenetic tree in Newick format used in PGLS and PGLMM analyses is available online at http://onlinelibrary.wiley.com/doi/10.1111/nph.13881/full

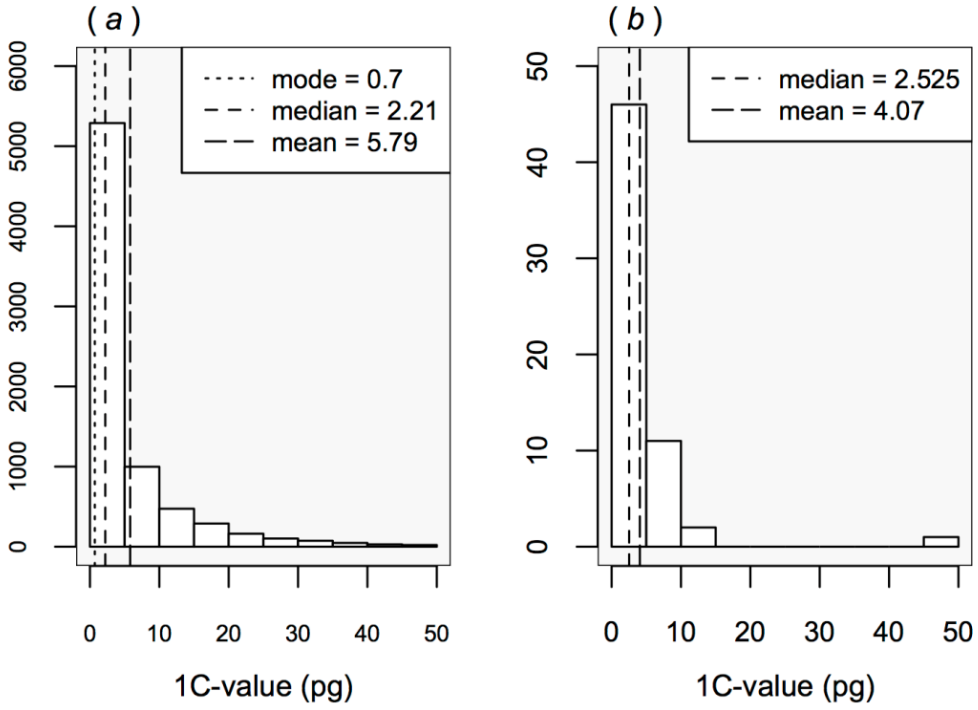


Figure S2. 1 Distribution of GS for (**a**) 7,484 angiosperms across 248 families, for species with 1C-values ≤ 50 pg. The mode, median and mean 1C-values (pg) for all 7,542 angiosperms listed in Bennett & Leitch (2012) are shown; (**b**) the 60 angiosperm species from 18 families on Park Grass, which range from 1C = 0.30 pg in *Carex flacca* to 1C = 47.3 pg in *Fritillaria meleagris*. Genome size data in (**a**) were obtained from Bennett MD, and Leitch IJ. 2012. Plant DNA C-values database, release 6.0, Dec. 2012. http://data.kew.org/cvalues.

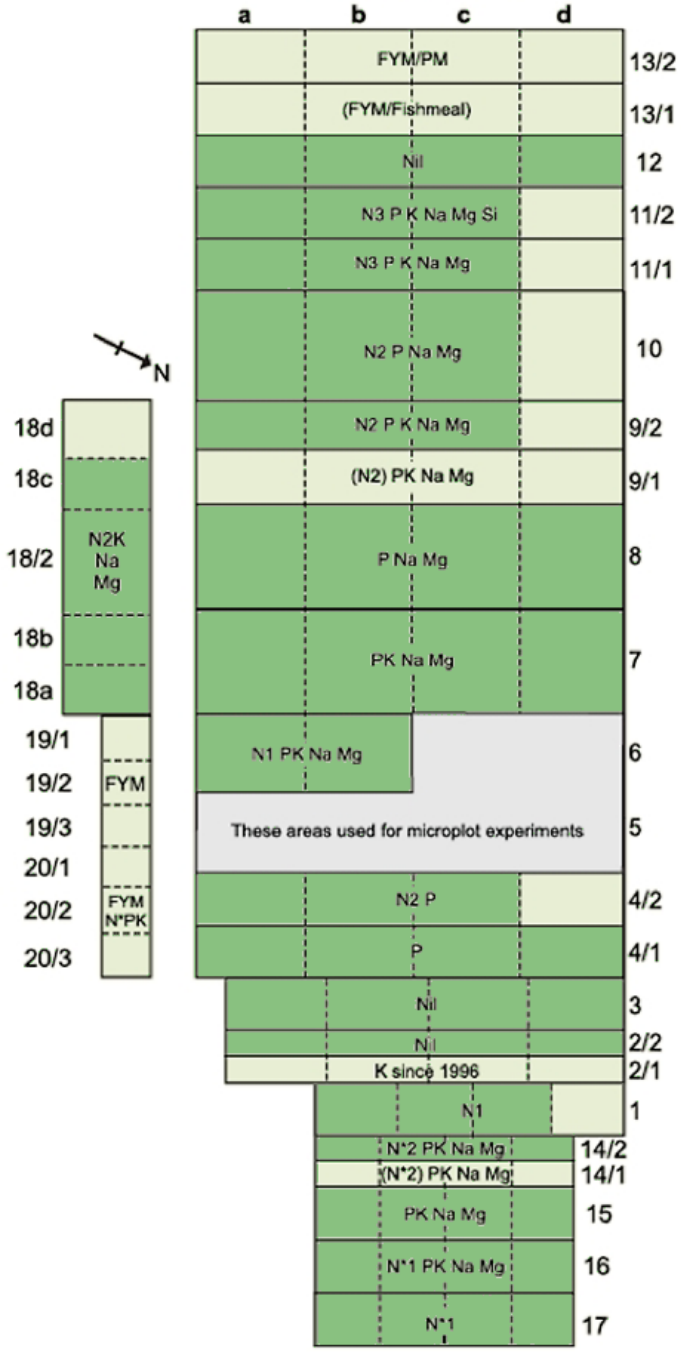


Figure S2. 2 Plot layout at Park Grass Experiment with fertilizer treatments (as it was when the herbage samples used in this paper were collected). We sampled 64 subplots, highlighted in dark green; these are subplots where macronutrient treatments have remained constant for at least a century. On these subplots, 15 combinations of N, P, K, Na, Mg, and Si are applied. Abbreviations are as follows: N = $(NH_4)_2SO_4$; N* = $NaNO_3$; where 1, 2 and 3 correspond to 48,

96 and 144 kg of N applied per hectare per year respectively. The other nutrients are applied annually at the following dosages: P, 35 kg ha^{-1}; K, 225 kg ha^{-1}; Na, 15 kg ha^{-1}; Mg, 10 kg ha^{-1} (Na and Mg always added together); and Si, 450 kg ha^{-1}. Abbreviations are as follows: P = P_2O_5; K = K_2SO_4; Mg = $MgSO_4$; Na = Na_2SO_4, Si = Na_2O_3Si. Two control plots receiving no nutrient treatments were established in 1856 (plots 3 and 12) and a third one (plot 2/2) was established in 1864. In 1903 most of the plots were divided into two to test the effects of lime ($CaCO_3$, 4 t ha^{-1}) applied every four years to the southern halves. In 1965 the plots were divided again into four smaller subplots (a, b, c and d), with subplots a, b and c receiving lime, every three years, to maintain the soil pH at 7, 6, and 5 respectively. The fourth subplot (d) remains unlimed and soil pH here varies from pH 3.6 (on subplots receiving N as $(NH_4)_2SO_4$) to pH 5.7 (on subplots receiving N as $NaNO_3$); control plots (receiving no treatments) are at c. pH 5.1. The herbage on each plot is cut annually in mid-late June and again in autumn. The plots were originally cut by scythe, then by horse-drawn and then tractor-drawn mowers. Yields were originally estimated by weighing the produce from the whole plot, either as hay (1st harvest) or green crop (2nd harvest), and dry matter determined. Since 1960, yields of dry matter have been estimated from strips cut with a forage harvester. However, for the first cut the remainder of each plot is still mown and made into hay, continuing earlier management and ensuring the return of seed. For the second cut, the whole of each plot is cut with a forage harvester. For more information on the Park Grass Experiment and recent changes in fertilizer treatments, see:

http://www.era.rothamsted.ac.uk/index.php?area=home&page=index&dataset=1.

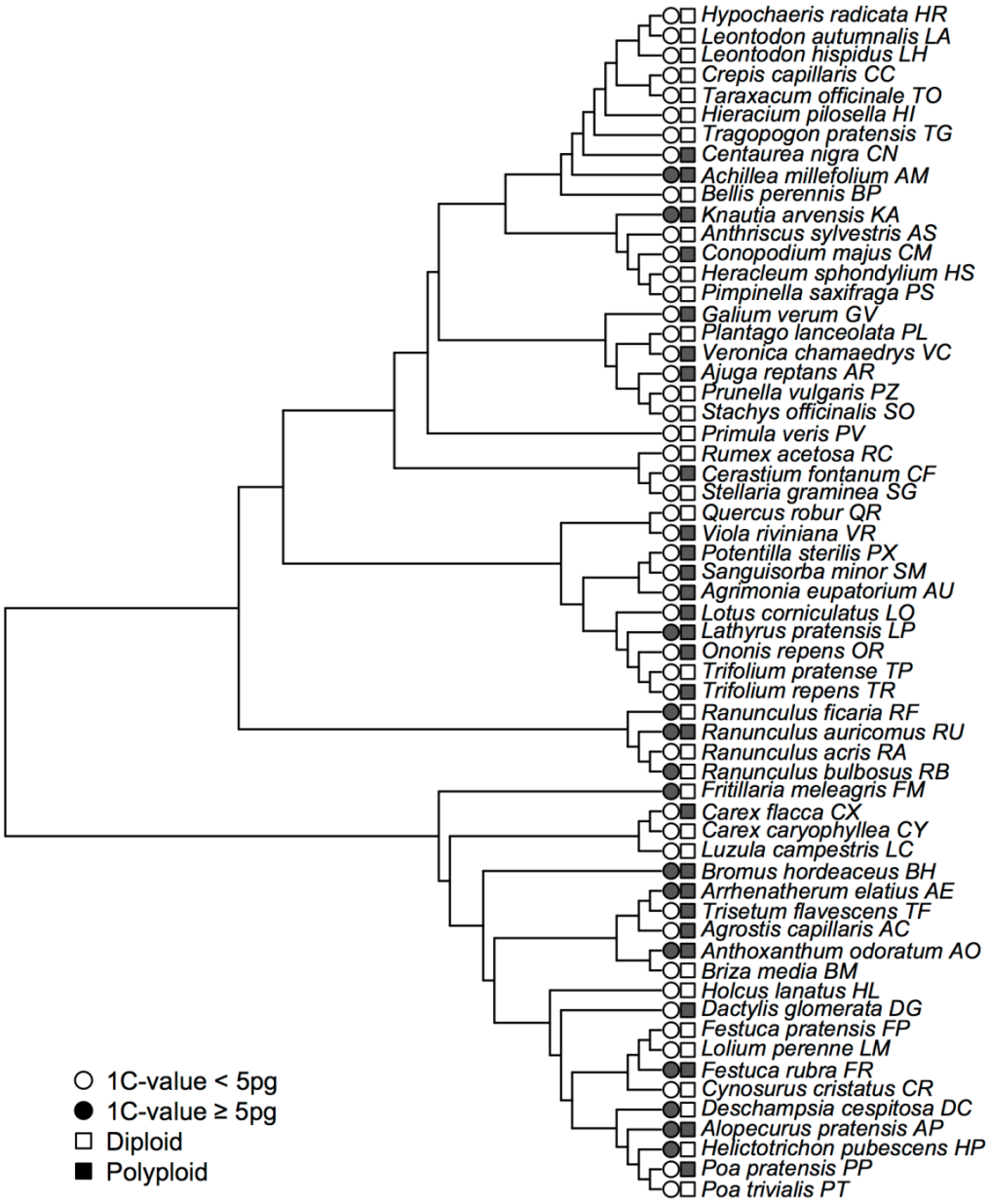


Figure S2. 3 Maximum likelihood phylogenetic tree of 60 angiosperm species present at the Park Grass experimental plots which was used to obtain a co-variance matrix for fitting linear regressions, phylogenetic independent contrasts and phylogenetic generalised linear mixed effect models. Taxa abbreviations are given next to the full taxa name. Taxon markers show whether the 1C-value < 5 pg, or ≥ 5 pg; and whether a taxon is diploid or polyploid. See also Methods S2.1.

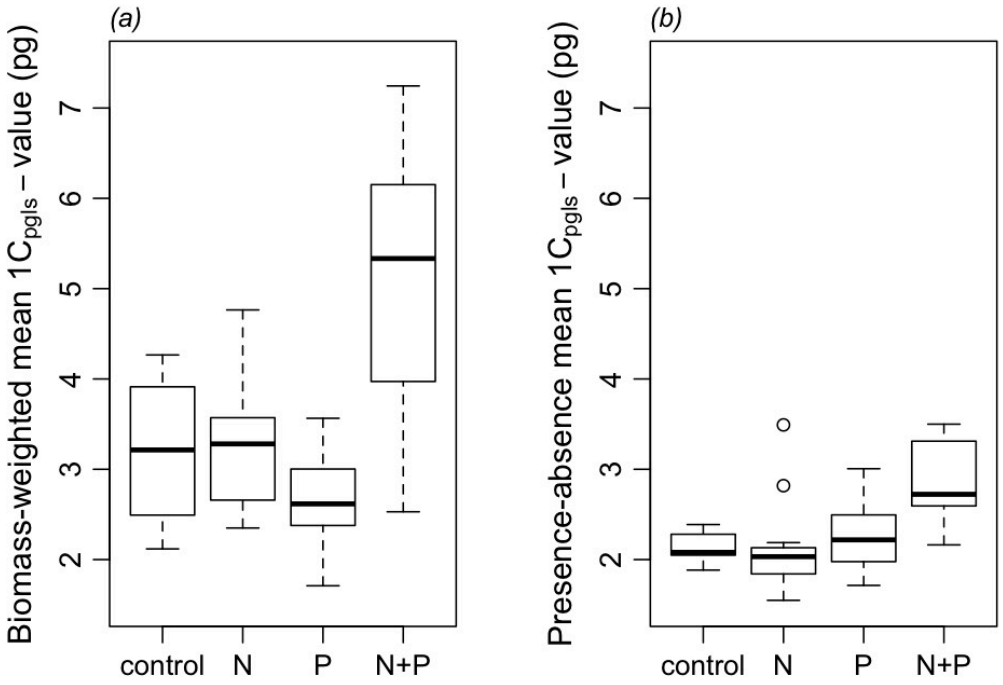


Figure S2. 4 Boxplots showing (**a**) biomass-weighted mean $1C_{pgls}$-values (i.e. phylogeny-adjusted GS weighted by species biomass); (**b**) presence-absence mean $1C_{pgls}$ -values (i.e. phylogeny-adjusted GS unweighted by biomass). See also legend to Table S2.4. pgls = phylogenetic generalised least squares.

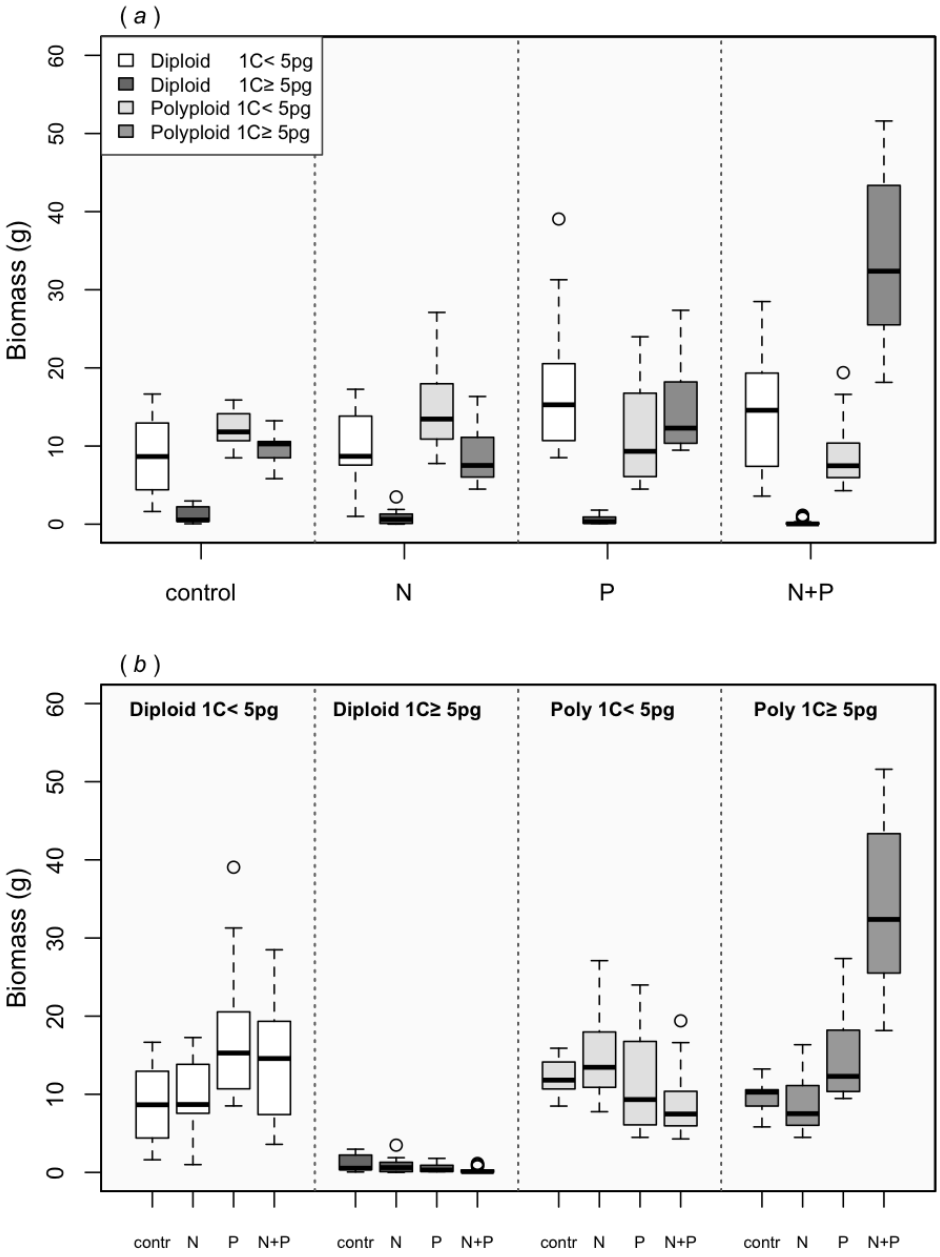


Figure S2. 5 Boxplots are shown here to facilitate comparison between the four genomic groups of: i) diploid taxa with small GS (1C-value < 5 pg); ii) diploid taxa with large GS (1C-value ≥ 5 pg); iii) polyploids with small GS; and, iv) polyploids with large GS; their mean total biomass is shown in boxplots by (**a**) treatment, and (**b**) by genomic group. "contr" = control (i.e. no nutrients), "Poly" = polyploid taxa.

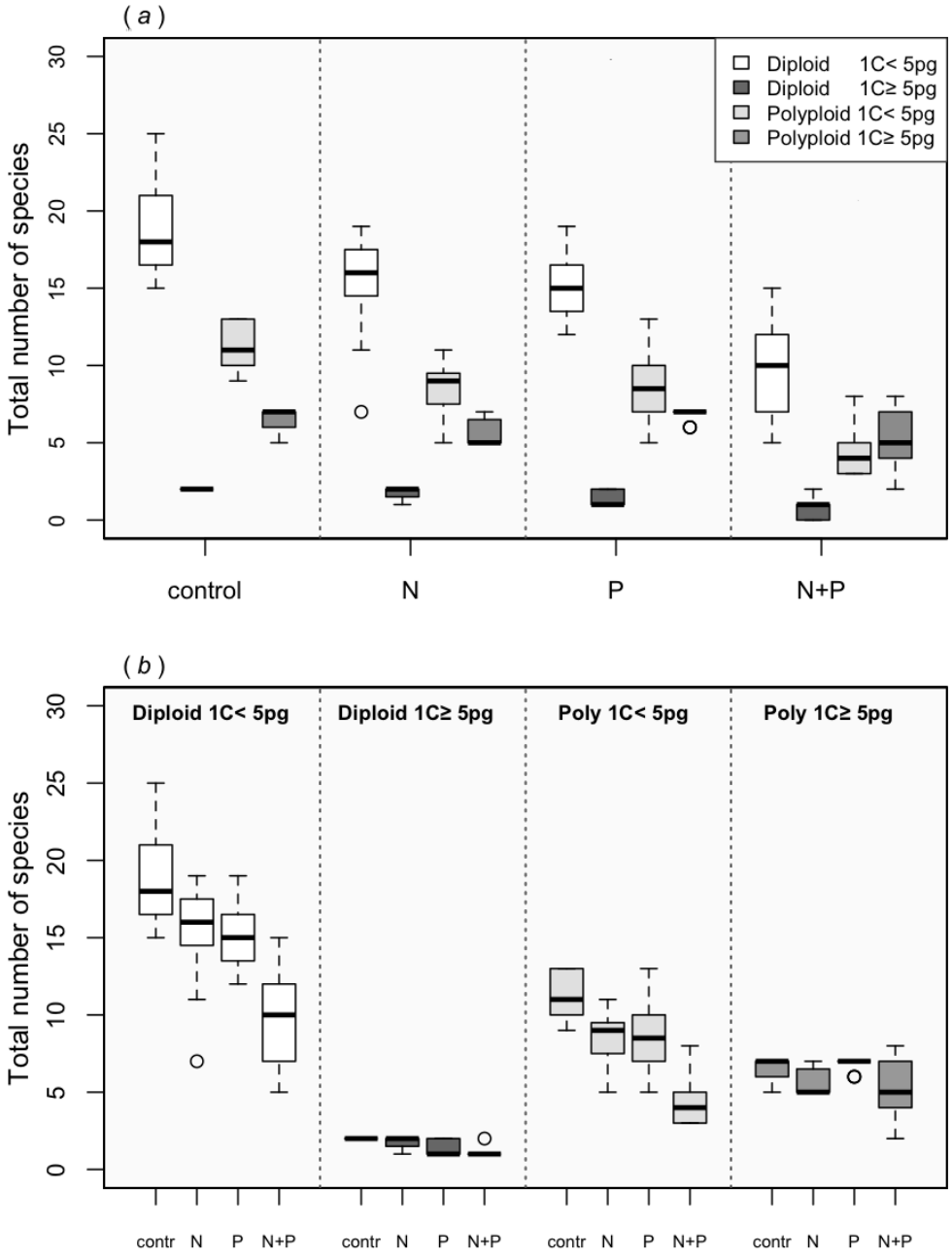


Figure S2. 6 Boxplots show the comparisons between the four genomic groups of: i) diploid taxa with small GS (1C-value < 5 pg); ii) diploid taxa with large GS (1C-value ≥ 5 pg); iii) polyploids with small GS; and iv) polyploids with large GS; the total number of species is shown in boxplots by (**a**) treatment, and (**b**) by genomic group. "contr" = control (i.e. no nutrients), "Poly" = polyploid taxa

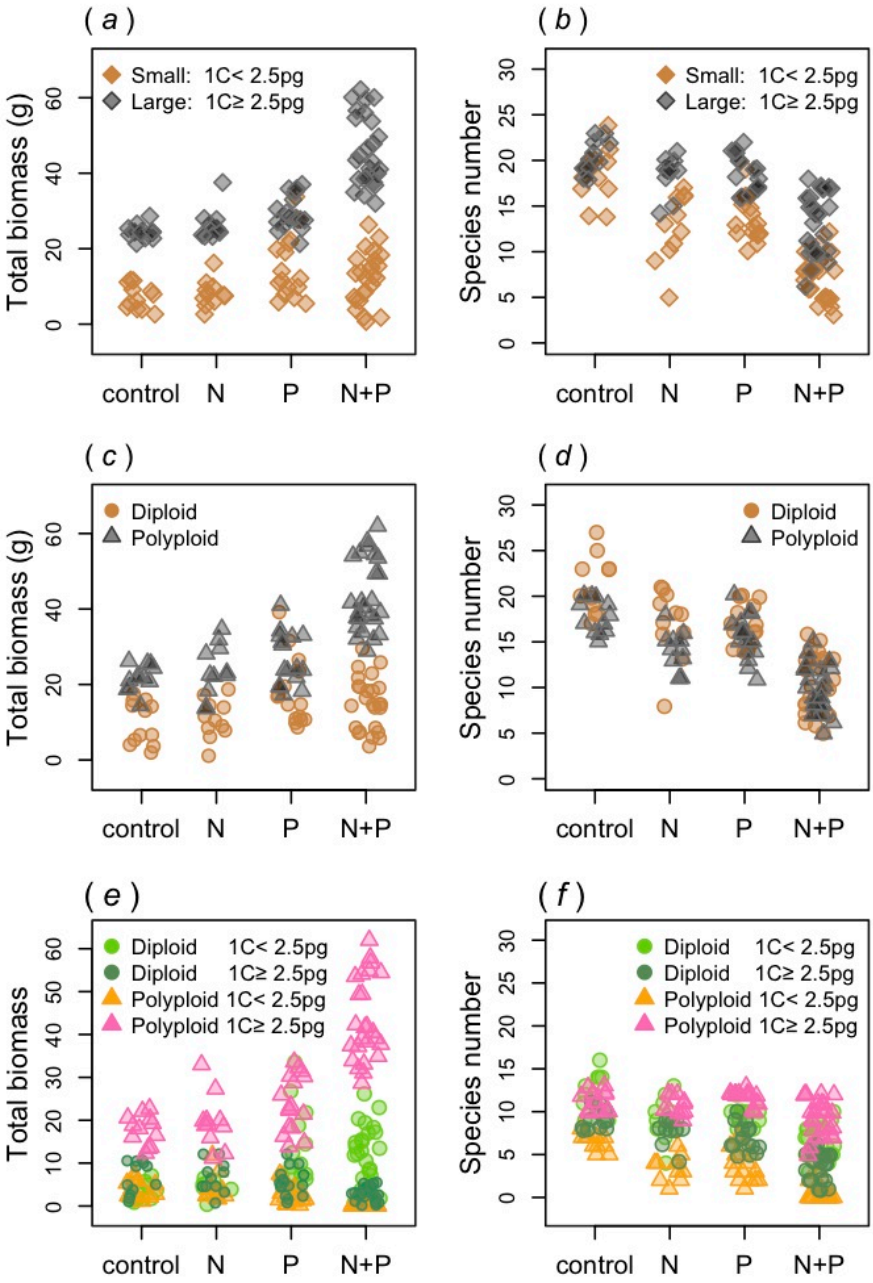


Figure S2. 7 Large GS ≥ 2.5 pg. Scatter plots comparing total biomass and species numbers between taxa with (**a**), (**b**) small vs large GS; (**c**), (**d**) diploid vs polyploid taxa; and (**e**), (**f**) the four groups based on GS and ploidy level: i) diploid taxa with small GS (1C-value < 2.5 pg); ii) diploid taxa with large GS (1C-value ≥ 2.5 pg); iii) polyploids with small GS; and, iv) polyploids with large GS. See also Tables S2.7, S2.8.

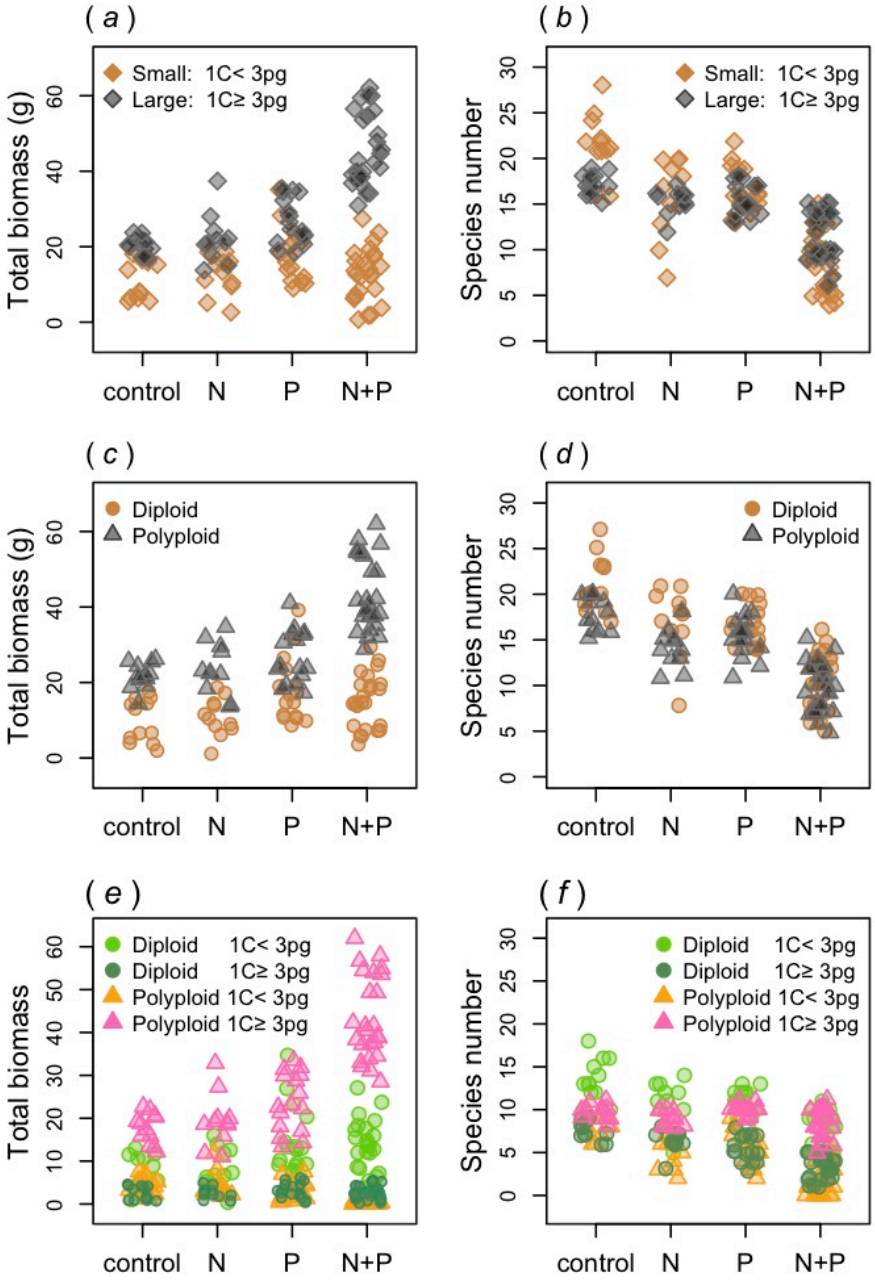


Figure S2. 8 Large GS ≥ 3 pg. Scatter plots comparing total biomass and species numbers between taxa with (**a**), (**b**) small vs large GS; (**c**), (**d**) diploid vs polyploid taxa; and (**e**), (**f**) the four groups based on GS and ploidy level: i) diploid taxa with small GS (1C-value < 3 pg); ii) diploid taxa with large GS (1C-value ≥ 3 pg); iii) polyploids with small GS; and, iv) polyploids with large GS. See also Tables S2.9, S2.10.

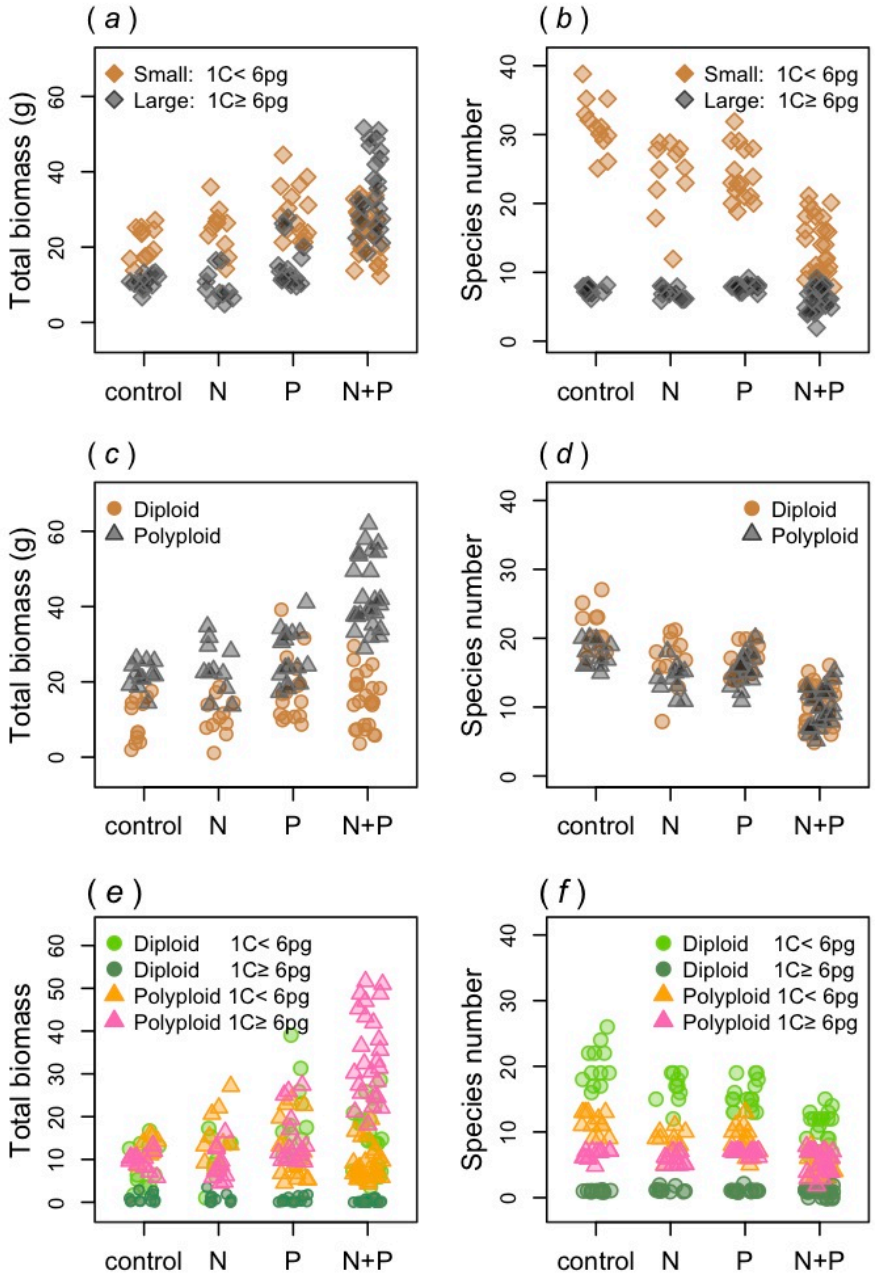


Figure S2. 9 Large GS ≥ 6 pg. Scatter plots comparing total biomass and species numbers between taxa with (**a**), (**b**) small vs large GS; (**c**), (**d**) diploid vs polyploid taxa; and (**e**), (**f**) the four groups based on GS and ploidy level: i) diploid taxa with small GS (1C-value < 6 pg); ii) diploid taxa with large GS (1C-value ≥ 6 pg); iii) polyploids with small GS; and, iv) polyploids with large GS. See also tables S2.11, S2.12.

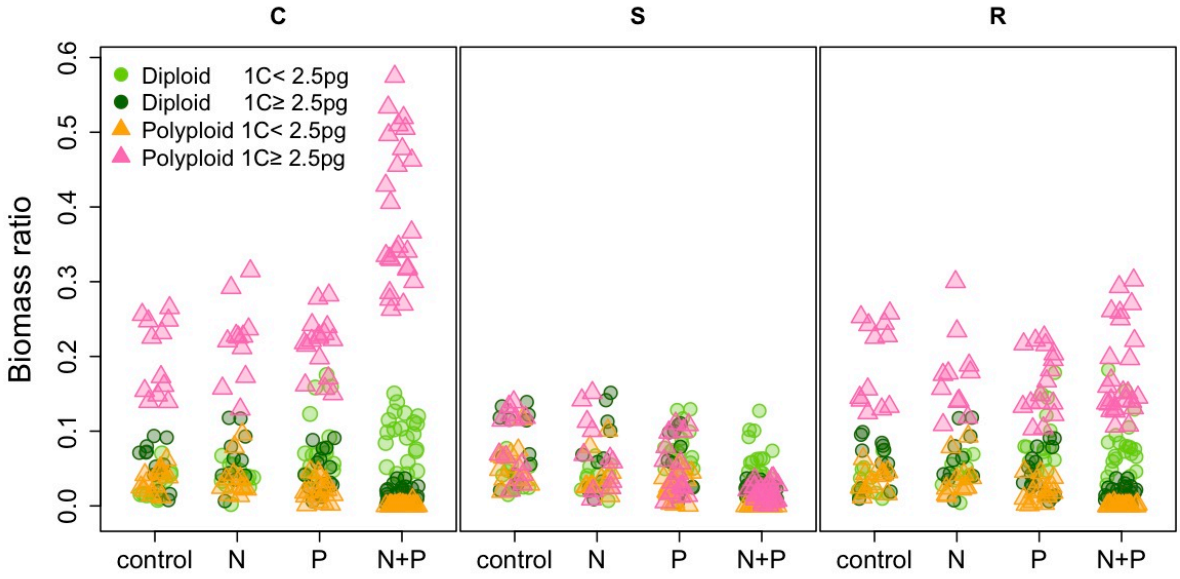


Figure S2. 10 Large GS ≥ 2.5 pg. Species above-ground biomass weighted by C-S-R strategy (C, competitor; S, stress-tolerant; R, ruderal). Each subplot is represented by four points corresponding to the four genomic parameter groups: i) diploids 1C-value < 2.5pg; ii) diploids 1C-value ≥ 2.5pg; iii) polyploids 1C-value < 2.5pg; and, iv) polyploids 1C-value ≥ 2.5pg. Polyploids with GS > 2.5pg were absent from 18 out of 24 N+P subplots (biomass ratio =0). See also Tables S2.7, S2.8.

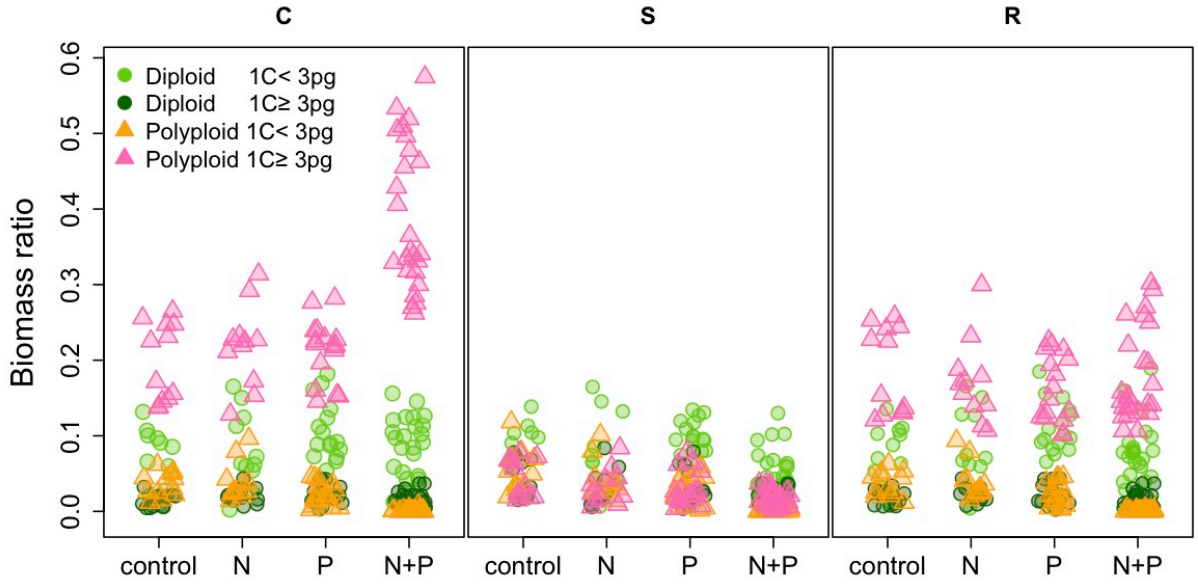


Figure S2. 11 Large GS ≥ 3 pg. Species biomass weighted by C-S-R strategy (C, competitor; S, stress-tolerant; R, ruderal), with a 3 pg threshold grouping taxa with large GS. Polyploids with GS ≥ 3pg were absent from nine out of 24 N+P subplots (biomass ratio =0). See also tables S2.9, S2.10.

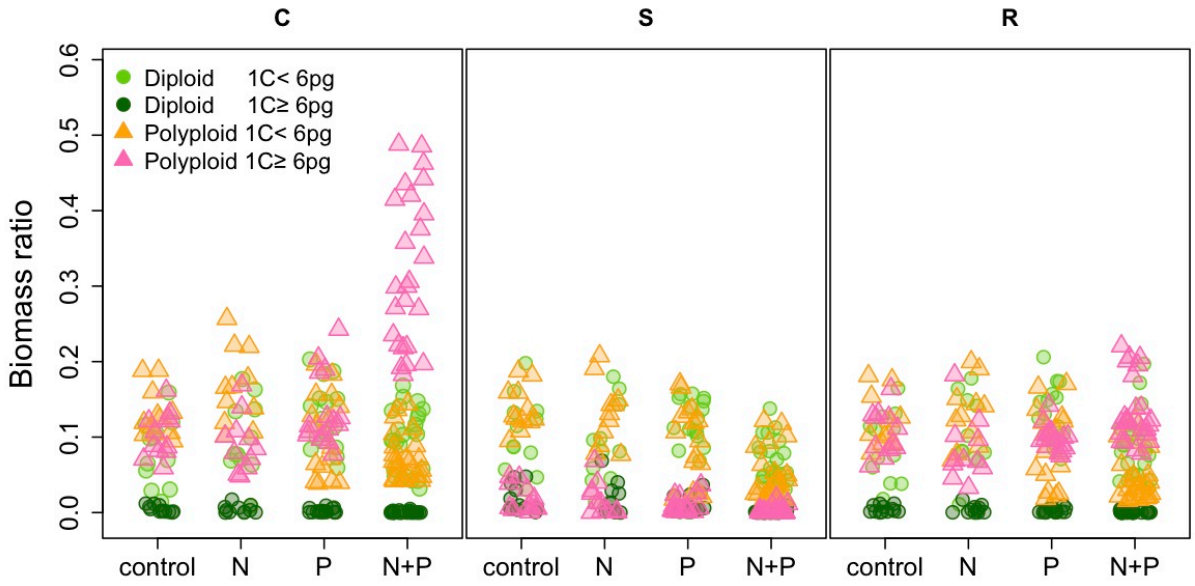


Figure S2. 12 Large GS threshold ≥ 6pg: Species biomass weighted by C-S-R strategy (C, competitor; S, stress-tolerant; R, ruderal), where the large GS threshold is set at 6 pg: i) diploids 1C-value < 6pg; ii) diploids 1C-value ≥ 6p, iii) polyploids 1C-value < 6pg; and, iv) polyploids 1C-value ≥ 6pg. Diploids with GS ≥ 6pg were absent from seven N+P subplots. (biomass ratio = 0). See also Tables S2.11, S2.12.

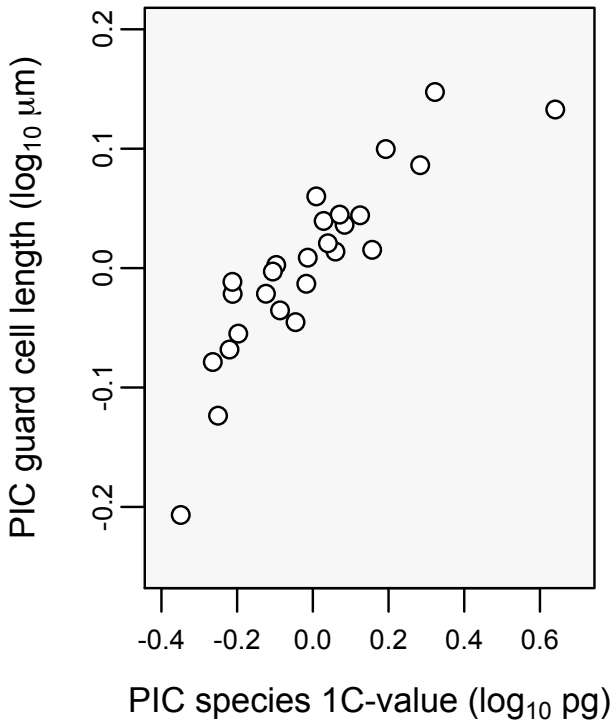


Figure S2. 13 Phylogeny-independent contrasts (PIC) on mean guard cell length and 1C-value in 27 taxa collected from the Park Grass Experiment plots. $R^2 = 0.761$, $F(1, 25)= 79.702$, $p<0.00001$.

Leaf imprints were made with clear varnish on fresh mature leaves and mounted on microscope slides. The length of 30 guard cells from leaves of two to 11 plants of each species were measured using OpenLab software and mean length estimated. Cell length and GS were log_{10} transformed and the association between PICs of guard cell sizes and PICs of 1C-values were tested in a linear regression through the origin with 10,000 permutations (functions *pic* and *lmorigin* from the ape package, Paradis et al. 2004).

Table S2. 1 Full list of species occurring in the 64 subplots analysed at Park Grass together with family and the accession numbers of the sequences obtained from NCBI's GenBank to estimate a phylogenetic tree.

ID	Species	Family	matK	rbcL	trnF-trnL	trnT-trnL	atpF-atpH
AM	*Achillea millefolium*	Asteraceae	HM850607.1	JX848399.1	AY603266.1	-	FJ395299.1
AU	*Agrimonia eupatorium*	Rosaceae	HM850683.1	JN891277.1	-	GQ384718.1	FJ395318.1
AC	*Agrostis capillaris*	Poaceae	JN895337.1	JN891522.1	EU119354.1	AY450936.1	FJ395329.1
AR	*Ajuga reptans*	Lamiaceae	AY840130.1	U32163.1	HQ911712.1	-	-
AP	*Alopecurus pratensis*	Poaceae	HM850564.1	HM849759.1	EU434101.1	-	EU434165.1
AO	*Anthoxanthum odoratum*	Poaceae	HM850562.1	HM849780.1	EF137590.1	-	FJ395289.1
AS	*Anthriscus sylvestris*	Apiaceae	U58547.1	JN893702.1	-	-	FJ395283.1
AE	*Arrhenatherum elatius*	Poaceae	EU434292.1	AY395529.1	-	DQ336866.1	FJ766100.1
BP	*Bellis perennis*	Asteraceae	HM850613.1	AY395530.1	JN315894.1	-	-
BM	*Briza media*	Poaceae	JN894143.1	AJ746285.1	DQ631446.1	DQ631512.1	-
BH	*Bromus hordeaceus*	Poaceae	HM850582.1	HM849826.1	EU036174.1	EU036148.1	GQ247913.1
CY	*Carex caryophyllea*	Cyperaceae	JN895022.1	JN892138.1	EU288430.1	-	-
CX	*Carex flacca*	Cyperaceae	JN895262.1	JN891463.1	DQ998968.1	-	-
CN	*Centaurea nigra*	Asteraceae	JN895499.1	JN893384.1	-	-	FJ395314.1
CF	*Cerastium fontanum*	Caryophyllaceae	HM850786.1	HM849881.1	AY521370.1	-	FJ395279.1
CM	*Conopodium majus*	Apiaceae	JN895810.1	JN893624.1	-	-	-
CC	*Crepis capillaris*	Asteraceae	JN895402.1	JN892652.1	-	-	-
CR	*Cynosurus cristatus*	Poaceae	HM850529.1	EF125151.1	EF137599.1	-	-
DG	*Dactylis glomerata*	Poaceae	HM850569.1	AY395535.1	AF533028.1	DQ631481.1	FJ395298.1
DC	*Deschampsia cespitosa*	Poaceae	JN894900.1	JX848495.1	AY237914.1	DQ631507.1	FJ395310.1
FP	*Festuca pratensis*	Poaceae	HM850535.1	JN891048.1	-	GU726883.1	-
FR	*Festuca rubra*	Poaceae	HQ593297.1	JN891471.1	-	DQ336857.1	FJ395301.1

Table S2.1 continued

ID	Species	Family	matK	rbcL	trnF-trnL	trnT-trnL	atpF-atpH
FM	*Fritillaria meleagris*	Liliaceae	AY624445.1	AY395537.1	-	-	-
GV	*Galium verum*	Rubiaceae	JN893877.1	JN892891.1	-	-	HQ594712.1
HP	*Helictotrichon pubescens*	Poaceae	JN895846.1	JN891447.1	-	DQ631526.1	-
HS	*Heracleum sphondylium*	Apiaceae	JN894476.1	JN893491.1	-	AM998530.1	FJ395304.1
HI	*Hieracium pilosella*	Asteraceae	HE970711.1	JN891685.1	DQ460865.1	-	HQ594728.1
HL	*Holcus lanatus*	Poaceae	JN894527.1	JN892327.1	EF137606.1	DQ631503.1	FJ395300.1
HR	*Hypochaeris radicata*	Asteraceae	HM850666.1	HM850069.1	AF528380.1	-	FJ395296.1
KA	*Knautia arvensis*	Dipsacaceae	JN895237.1	JN892433.1	FJ640666.1	-	-
LP	*Lathyrus pratensis*	Fabaceae	JX505811.1	JN891335.1	JX505683.1	-	-
LA	*Leontodon autumnalis*	Asteraceae	JN895402.1	JN892652.1	AF528391.1	-	-
LH	*Leontodon hispidus*	Asteraceae	JN894007.1	JN890753.1	JQ041846.1	-	-
LM	*Lolium perenne*	Poaceae	HM850533.1	JN893059.1	EU119376.1	DQ367404.1	FJ766122.1
LO	*Lotus corniculatus*	Fabaceae	HM049505.1	JN892127.1	-	-	HQ594766.1
LC	*Luzula campestris*	Juncaceae	JN895446.1	HM850146.1	AY437943.1	-	FJ395316.1
OR	*Ononis repens*	Fabaceae	JN895637.1	JN890867.1	-	-	-
PS	*Pimpinella saxifraga*	Apiaceae	FR865050.1	JN892078.1	-	-	-
PL	*Plantago lanceolata*	Plantaginaceae	HE966968.1	JN893615.1	EU036272.1	-	GQ248030.1
PP	*Poa pratensis*	Poaceae	JN966444.1	JN965752.1	-	JF904790.1	FJ395325.1
PT	*Poa trivialis*	Poaceae	HM850517.1	JN893080.1	AY327795.1	-	FJ395264.1
PX	*Potentilla sterilis*	Rosaceae	JN895651.1	JN893010.1	FN561732.1	-	-
PV	*Primula veris*	Primulaceae	JN896058.1	AF394982.1	JQ927136.1	-	-
PZ	*Prunella vulgaris*	Lamiaceae	HM850805.1	AY395556.1	AY506619.1	-	-
QR	*Quercus robur*	Fagaceae	JN895518.1	FN675735.1	HM770040.1	-	-
RA	*Ranunculus acris*	Ranunculaceae	JN894744.1	JN965795.1	-	-	FJ395343.1

Table S2.1 continued

ID	Species	Family	matK	rbcL	trnF-trnL	trnT-trnL	atpF-atpH
RU	*Ranunculus auricomus*	Ranunculaceae	JN894692.1	JN893758.1	-	-	-
RB	*Ranunculus bulbosus*	Ranunculaceae	HM851057.1	JN892326.1	FJ490812.1	-	FJ395281.1
RF	*Ranunculus ficaria*	Ranunculaceae	AY954232.1	EU053919.1	-	-	-
RC	*Rumex acetosa*	Polygonaceae	JN895619.1	JN893396.1	AJ583853.1	-	FJ395278.1
SM	*Sanguisorba minor*	Rosaceae	HM850691.1	JN892329.1	EU873351.1	-	-
SO	*Stachys officinalis*	Lamiaceae	JN896053.1	HE963693.1	FJ854224.1	-	-
SG	*Stellaria graminea*	Caryophyllaceae	JN895064.1	JN892194.1	AY521345.1	-	-
TO	*Taraxacum officinale*	Asteraceae	FJ395377.1	JX520956.1	EF015611.1	-	FJ395276.1
TG	*Tragopogon pratensis*	Asteraceae	JN893953.1	JN890681.1	JQ041858.1	JQ041828.1	FJ395355.1
TP	*Trifolium pratense*	Fabaceae	JN895372.1	JN893083.1	JQ041859.1	-	FJ395288.1
TR	*Trifolium repens*	Fabaceae	HE967014.1	JN892960.1	AB546814.1	-	FJ395344.1
TF	*Trisetum flavescens*	Poaceae	JN895340.1	JN893258.1	DQ336850.1	DQ336877.1	-
VC	*Veronica chamaedrys*	Plantaginaceae	JN894843.1	JN891876.1	AF486377.1	-	-
VR	*Viola riviniana*	Violaceae	JN894328.1	JN893557.1	-	-	-

Table S2. 1 Full list of taxa occurring at Park Grass with GS (1C-values), chromosome number (2n), ploidy level (x), and C-S-R type, organized according to genomic grouping: Diploids with 1C-value < 5pg, diploids with 1C-value ≥ 5pg, polyploids with 1C-value < 5pg, and polyploids with 1C-value ≥ 5pg. Where GS estimates were obtained for the present work, the standard deviation is given (SD) together with the mean coefficient of variation (CoV, %)‡ for the flow histogram peaks of both the target taxon and the internal standard. The number of plants we sampled to estimate GS are listed under the column heading (n). Other GS estimates were taken from Bennett & Leitch (2012) and are listed in the "Ref" column; see below this table for the authors. Where our CoV was high (e.g. > 4), and if available, we used a previously published C-value that was closest to ours, and give our 1C-value estimate in the SD column (along with the SD). Overall, our dataset comprises: 29 diploid species with small GS (1C < 5 pg) (3 monocot and 9 eudicot families); 5 diploid species with big GS (1C ≥ 5 pg) (2 monocot and 1 eudicot family); 17 polyploid species with small GS (2 monocot and 8 eudicot families); and 9 polyploid species with big GS (1 monocot and 4 eudicot families). "Chrom. num" = chromosome number.

ID	Species	Family	1C-value (pg)	1C-value sd	Target taxon CoV‡	Internal standard CoV ‡	n	Chrom. num	Ploidy level (x)	CSR type	*Ref
Diploid 1C < 5 pg											
AS	*Anthriscus sylvestris*	Apiaceae	2.25	2.18 ±0.04	5.27 ±1.11	2.32 ±0.24	3	16	2	C/CR	2
BM	*Briza media*	Poaceae	3.35	0.07	2.29 ±0.97	2.43 ±0.86	19	na	2	S/CSR	-
BP	*Bellis perennis*	Asteraceae	1.15	-	-	-	-	18	2	R/CSR	13
CC	*Crepis capillaris*	Asteraceae	2.10	-	-	-	-	6	2	R/SR	7
CM	*Conopodium majus*	Apiaceae	0.83	0.02	4.15 ±0.5	2.74 ±0.44	6	22	2	SR	-
CR	*Cynosurus cristatus*	Poaceae	3.05	-	-	-	-	14	2	R/CSR	18
CY	*Carex caryophyllea*	Cyperac.	0.78	-	-	-	-	66	2	S/CSR	11
FP	*Festuca pratensis*	Poaceae	2.23	-	-	-	-	14	2	CSR	16
HI	*Hieracium pilosella*	Asteraceae	3.53	0.06	2.8 ±0.31	2.59 ±0.67	8	18	2	S/CSR	-
HL	*Holcus lanatus*	Poaceae	1.89	0.12	2.48 ±0.67	2.16 ±0.54	50	14	2	CSR	-
HR	*Hypochaeris radicata*	Asteraceae	1.34	-	-	-	-	8	2	CSR	6
HS	*Heracleum sphondylium*	Apiaceae	2.19	2.56 ±0.13	3.42 ±0.80	2.78 ±0.18	2	22	2	C/CSR	17
LA	*Leontodon autumnalis*	Asteraceae	1.16	-	-	-	-	12	2	R/CSR	17

Table S2.2 continued

ID	Species	Family	1C-value (pg)	1C-value sd	Target taxon CoV	Internal standard CoV	n	Chrom. num	Ploidy level (x)	CSR type	*Ref
LC	*Luzula campestris*	Juncaceae	0.43	0.02	3.89 ±0.77	2.62 ±0.76	2	12	2	S/CSR	-
LH	*Leontodon hispidus*	Asteraceae	2.50	0.07	4.51 ±0.09	2.17 ±0.1	2	14	2	CSR	5
LM	*Lolium perenne*	Poaceae	3.06	0.03	3.23 ±0.3	3.12 ±0.17	3	14	2	CR/CSR	-
PL	*Plantago lanceolata*	Plantagin.	1.20	-	4.09 ±NA	3.51 ±NA	1	12	2	CSR	11
PS	*Pimpinella saxifraga*	Apiaceae	3.89	0.04	4.63 ±2.22	3.13 ±0.76	3	20	2	SR/CSR	-
PT	*Poa trivialis*	Poaceae	1.85	0.03	4.17 ±1.15	2.72 ±0.94	13	14	2	R/CSR	-
PV	*Primula veris*	Primula.	0.49	-	-	-	-	22	2	S/CSR	17
PZ	*Prunella vulgaris*	Lamiaceae	0.65	-	-	-	-	28	2	CSR	23
QR	*Quercus robur*	Fagaceae	0.93	-	-	-	-	24	2	SC	8
RA	*Ranunculus acris*	Ranuncul.	4.74	0.19	3.8 ±1.01	2.92 ±0.7	21	14	2	CSR	-
RC	*Rumex acetosa*	Polygon.	3.55	0.07	2.28 ±0.56	2.39 ±0.41	7	14	2	CSR	-
SG	*Stellaria graminea*	Caryophyll.	1.17	NA	3.13	2.61	1	26	2	CSR	-
SO	*Stachys officinalis*	Lamiaceae	4.53	4.91 ±NA	4.19 ±NA	3.3 ±NA	1	16	2	S/CSR	2
TG	*Tragopogon pratensis*	Asteraceae	2.77	2.53 ±NA	4.93 ±NA	2.03 ±NA	1	12	2	CR/CSR	14
TO	*Taraxacum officinale*	Asteraceae	1.53	0.28	3.77 ±1.14	2.89 ±1	18	16	2	R/CSR	-
TP	*Trifolium pratense*	Fabaceae	0.53	0.51 ±NA	3.17 ±NA	2.11 ±NA	1	14	2	CSR	3
Diploid 1C ≥ 5 pg											
DC	*Deschampsia cespitosa*	Poaceae	5.22	-	-	-	-	26	2	SC/CSR	12
FM	*Fritillaria meleagris*	Liliaceae	47.30	-	-	-	-	24	2	SR	10
HP	*Helictotrichon pubescens*	Poaceae	6.28	-	-	-	-	14	2	S/CSR	15
RB	*Ranunculus bulbosus*	Ranuncul.	5.63	-	-	-	-	16	2	SR	19
RF	*Ranunculus ficaria*	Ranuncul.	9.33	-	-	-	-	16	2	SR	20
Polyploid < 5 pg											
AC	*Agrostis capillaris*	Poaceae	3.53	-	-	-	15	28	4	CSR	11
AR	*Ajuga reptans*	Lamiaceae	1.19	0.04	3.09 ±0.75	2.89 ±0.61	9	32	poly	R/CSR	-
AU	*Agrimonia eupatorium*	Rosaceae	3.98	0.01	3.33 ±0.34	3.04 ±0.42	3	28	poly	CSR	-
CF	*Cerastium fontanum*	Caryophyll.	2.93	-	-	-	-	144	8, 16	R/CSR	4

Table S2.2 continued

ID	Species	Family	1C-value (pg)	1C-value sd	Target taxon CoV	Internal standard CoV	n	Chrom. num	Ploidy level (x)	CSR type	*Ref
CN	*Centaurea nigra*	Asteraceae	2.25	0.04	3.11 ±0.57	2.56 ±0.42	4	44	poly	CSR	-
CX	*Carex flacca*	Cyperaceae	0.30	-	-	-	-	76	4	S/SC	9
DG	*Dactylis glomerata*	Poaceae	4.15	0.11	4.38 ±1.28	3.62 ±0.74	9	28	4	C/CSR	-
GV	*Galium verum*	Rubiaceae	1.89	2.10	5.22	2.79	5	44	4	SC/CSR	23
LO	*Lotus corniculatus*	Fabaceae	1.27	0.08	3.78 ±0.38	3.37 ±1.13	24	24	4	S/CSR	-
OR	*Ononis repens*	Fabaceae	1.41	0.01	4.79 ±0.79	3.16 ±0.52	6	various	poly	SC/CSR	-
PP	*Poa pratensis*	Poaceae	4.90	1.08	3.33 ±1.12	3.46 ±0.9	31	various	poly	CSR	-
PX	*Potentilla sterilis*	Rosaceae	0.97	-	-	-	1	28	poly	SR/CSR	-
SM	*Sanguisorba minor*	Rosaceae	0.55	0.62 ±NA	4.85 ±NA	2.93 ±NA	1	28	4	S/CSR	9
TF	*Trisetum flavescens*	Poaceae	2.55	-	-	-	-	various	poly	CSR	2
TR	*Trifolium repens*	Fabaceae	1.29	0.22	2.41 ±0.31	2.06 ±0.35	8	32	4	CR/CSR	-
VC	*Veronica chamaedrys*	Plantagin.	1.49	-	-	-	-	32	4	CSR	1
VR	*Viola riviniana*	Violaceae	1.35	-	-	-	-	40	4	S/CSR	9
Polyploid ≥ 5 pg											
AE	*Arrhenatherum elatius*	Poaceae	7.99	0.22	2.82 ±0.87	3.09 ±0.46	31	28	4	C/CSR	-
AM	*Achillea millefolium*	Asteraceae	8.37	0.07	3.09 ±0.66	2.24 ±0.62	49	54	6	CSR	-
AO	*Anthoxanthum odoratum*	Poaceae	6.31	0.21	2.04 ±0.43	2.33 ±0.45	67	20	4	SR/CSR	-
AP	*Alopecurus pratensis*	Poaceae	6.80	-	-	-	-	28	4	C/CSR	13
BH	*Bromus hordeaceus*	Poaceae	11.06	0.06	2.99 ±0.78	2.97 ±0.69	5	28	4	R/CR	-
FR	*Festuca rubra*	Poaceae	6.13	0.14	2.02 ±0.52	2.45 ±0.47	45	42	6	CSR	-
KA	*Knautia arvensis*	Dipsaca.	7.01	7.09 ±0.23	3.31 ±0.45	2.96 ±0.16	10	40	4	CSR	22
LP	*Lathyrus pratensis*	Fabaceae	11.46	0.43	3.34 ±0.99	3.7 ±0.82	-	na	poly	CSR	-
RU	*Ranunculus auricomus*	Ranuncul.	9.00	-	-	-	-	32	4	SR	21

‡ The CoVs shown in the table are provided by the flow cytometry software, which returns a CoV for each peak (representing the standard + target taxon). A higher CoV indicates the estimate is noisier, less precise. Best practice in flow cytometry suggests that only genome size estimations with a CoV less than 5 (or even less than 3) be published as new GS estimations. Outside of equipment and laboratory practice issues, a high CoV is often caused by the presence of secondary metabolites within the leaf that interfere with flow cytometry buffers.

* Reference sources for the C-values used in the present work that were taken from the Plant DNA C-values database (Bennett and Leitch 2012). The database source references were:1) Albach & Greilhuber, 2003 (reference 478); 2) Band, 1984 (reference 154); 3) Barow & Meister, 2003 (reference 492); 4) Bennett, Smith & Lewis Smith., 1982 (reference 105); 5) Callimassia & Bennett, 1992 (reference 269-H); 6) Cerbah *et al.,* 1999 (reference 666); 7) Evans *et al.*, 1972 (reference 7); 8) Favre & Brown, 1996 (reference 366); 9) Grime *et al.*, 1985 (reference 133); 10) Leitch *et al.,* 2007 (reference 565); 11) Mowforth, 1986 (reference 158); 12) Murray *et al.*, 2005 (reference 528); 13) Olszewska & Osiecka, 1982 (reference 155); 13) Olszewska & Osiecka, 1983 (reference 156); 14) Pires *et al.,* 2004 (reference 510); 15) Roser, 1995 (reference 283); 16) Seal, 1979 (reference 72); 17) Siljak-Yakovlev *et al.*, 2010 (reference 634); 18) Šmarda *et al.,* 2008 (reference 555); 19) Smith & Bennett, 1975 (reference 45); 20) Smith & Bennett, 1975 (reference 45); 21) Smith & Bennett, 1975 (reference 45); 22) Temsch & Greilhuber, 2010 (reference 652); 23) Temsch *et al.,* 2010 (reference 627).

Table S2. 3 Measures of arithmetic mean and standard deviation under four different nutrient treatments: (control i.e. no fertilizer); N (N without P); P (P without N); N+P (both N and P applied) on: mean biomass (above-ground dry weight), biomass-weighted mean 1C-value, biomass-weighted mean $1C_{pgls}$ (phylogenetic least squares); presence-absence (i.e. unweighted by biomass) mean $1C_{pgls}$. n= number of subplots in each treatment. $1C_{pgls}$ means were log_{10} back-transformed to facilitate interpretation. $1C_{pgls}$ presence-absence are unweighted by biomass. Mean $1C_{pgls}$ were estimated as in Šmarda et al. (2013), using the gls function in the nlme package (Pinheiro et al., 2013) and the ape package (Paradis et al., 2004). Pgls accommodates models with phylogenetic correlation structures and we used it to obtain for each subplot a phylogeny-adjusted biomass–weighted mean GS (= 'biomass-weighted mean $1C_{pgls}$ -value') which takes into account each taxon's contribution to total biomass, and a presence-absence mean which is unweighted by species biomass. We calculated $1C_{pgls}$ by using the R formula y~1 to estimate subplot means where y = the 1C-value of each taxon; a within group structure was specified by Brownian motion phylogenetic covariance, and biomass fraction, or presence-absence, described the within-group heteroscedasticity. A phylogenetic tree of 60 species was used to estimate both types of mean $1C_{pgls}$ -value for each subplot (see Methods S2.1 for phylogenetic tree file). A biomass value of 0.000001 was attributed to absent species.

Treatment	*n*	Total biomass (g)	Spp no.	Biomass-weighted mean 1C (pg)	Biomass-weighted Mean $1C_{pgls}$ (pg)	Presence-absence mean $1C_{pgls}$ (pg)
Control	12	31.71 ±4.27	39 ±4.2	3.99 ±0.37	3.19 ±0.81	2.12 ±0.14
N	11	34.47 ±3.9	31 ±5.6	3.87 ±0.51	3.24 ±0.73	2.13 ±0.54
P	16	44.49 ±11.75	32 ±4.2	4.17 ±0.44	2.66 ±0.5	2.24 ±0.36
N+P	25	58.36 ±11.64	20 ±5.5	5.4 ±0.52	5.07 ±1.28	2.81 ±0.42

Table S2. 4 Means and ratios with standard deviations per nutrient treatment for:(**a**) total subplot biomass; (**b**) total number of species per subplot, and (**c**) total C-weighted; (**d**) total S-weighted; (**e**) total R-weighted biomass for the four genomic groups of taxa: diploid taxa with small GS; diploid taxa with big GS, polyploid taxa with small GS; and polyploid taxa with big GS. 1C= 1C-value (pg). The number of subplots per treatment is as follows: control=12, N=11, P=16, N+P= 25.

(a)	**Mean total biomass (g)**				**Mean ratio total biomass**			
Treatment	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg
Control	8.78 ±4.95	1.15 ±1.1	12.06 ±2.41	9.72 ±2.02	0.267 ±0.14	0.033 ±0.03	0.385 ±0.09	0.314 ±0.09
N	9.91 ±4.74	0.89 ±1.06	14.93 ±6.01	8.74 ±3.8	0.291 ±0.15	0.027 ±0.03	0.425 ±0.13	0.257 ±0.12
P	17.22 ±8.62	0.56 ±0.59	11.8 ±6.89	14.9 ±6.19	0.376 ±0.11	0.015 ±0.02	0.282 ±0.16	0.327 ±0.06
N+P	14.84 ±6.91	0.2 ±0.35	8.96 ±4.06	34.35 ±10.31	0.249 ±0.1	0.004 ±0.01	0.163 ±0.09	0.584 ±0.11

(b)	**Mean total number of species**				**Mean ratio total species number**			
Treatment	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg
Control	19 ±3	2 ±0	11 ±2	7 ±1	0.49 ±0.04	0.052 ±0.01	0.29 ±0.03	0.168 ±0.02
N	15 ±4	2 ±0	8 ±2	6 ±1	0.485 ±0.05	0.056 ±0.02	0.268 ±0.02	0.191 ±0.05
P	15 ±2	1 ±1	9 ±2	7 ±0	0.476 ±0.03	0.043 ±0.01	0.266 ±0.04	0.215 ±0.02
N+P	10 ±3	1 ±1	4 ±1	5 ±2	0.48 ±0.05	0.037 ±0.03	0.218 ±0.05	0.265 ±0.04

Table S2. 4 continued

(c)	C-weighted mean total biomass (g)				C-weighted mean ratio			
Treatment	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg
Control	2.64 ±1.57	0.16 ±0.17	4.069 ±0.85	3.185 ±0.67	0.08 ±0.04	0.005 ±0	0.13 ±0.03	0.103 ±0.03
N	3.336 ±1.59	0.152 ±0.17	5.836 ±2.39	3.138 ±1.23	0.098 ±0.05	0.005 ±0.01	0.165 ±0.05	0.092 ±0.04
P	5.819 ±3.37	0.092 ±0.1	4.361 ±2.45	6.294 ±3.90	0.125 ±0.04	0.002 ±0	0.102 ±0.05	0.132 ±0.05
N+P	5.918 ±3.04	0.034 ±0.06	4.134 ±1.83	19.548 ±9.10	0.097 ±0.04	0.001 ±0	0.072 ±0.03	0.32 ±0.1

(d)	S-weighted mean total biomass (g)				S-weighted mean ratio			
Treatment	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg
Control	3.316 ±1.77	0.733 ±0.72	4.312 ±0.86	3.268 ±0.68	0.101 ±0.05	0.021 ±0.02	0.137 ±0.03	0.106 ±0.03
N	3.068 ±1.63	0.589 ±0.71	4.574 ±1.86	2.793 ±1.29	0.091 ±0.05	0.018 ±0.02	0.131 ±0.04	0.082 ±0.04
P	5.422 ±2.13	0.373 ±0.39	3.801 ±2.23	4.291 ±1.27	0.121 ±0.03	0.01 ±0.01	0.092 ±0.05	0.097 ±0.02
N + P	3.718 ±1.72	0.136 ±0.23	2.416 ±1.41	7.342 ±1.59	0.065 ±0.03	0.003 ±0	0.046 ±0.03	0.131 ±0.04

(e)	R-weighted mean total biomass (g)				R-weighted mean ratio			
Treatment	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg	Diploid 1C< 5pg	Diploid 1C≥ 5pg	Polyploid 1C< 5pg	Polyploid 1C≥ 5pg
Control	2.822 ±1.63	0.252 ±0.22	3.68 ±0.86	3.268 ±0.68	0.086 ±0.04	0.007 ±0.01	0.118 ±0.03	0.106 ±0.03
N	3.502 ±1.6	0.151 ±0.18	4.518 ±1.83	2.812 ±1.31	0.102 ±0.05	0.005 ±0.01	0.129 ±0.04	0.083 ±0.04
P	5.979 ±3.16	0.099 ±0.1	3.638 ±2.27	4.316 ±1.3	0.13 ±0.04	0.003 ±0	0.087 ±0.05	0.098 ±0.02
N+P	5.208 ±2.67	0.034 ±0.06	2.412 ±1.41	7.464 ±1.65	0.087 ±0.04	0.001 ±0	0.046 ±0.03	0.133 ±0.04

Table S2. 5 Treatment contrasts and ANOVA output testing the effects and interactions of N, P, GS (small vs large, where large GS ≥ 5 pg), ploidy (diploid vs polyploid), on square-root transformed subplot dependent variables: (**a**) total biomass; (**b**) total species numbers; (**d**) C-weighted biomass; (**e**) S-weighted biomass; and (**f**) R-weighted biomass testing for significance in treatment, GS, and ploidy. Part (**c**) shows multivariate ANOVA output where each C-S-R-weighted biomass are the response variables (i.e. n=3). Baseline levels in the contrasts are: without N, without P, diploid, and small GS for N, P, GS and ploidy effects respectively (e.g. there is a 0.2 g increase in biomass with the addition of N, relative to subplots without N).

(a) Biomass

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	2.833	0.209	13.564	<0.0001	Intercept	1, 180	4171.593	<0.0001
N	0.202	0.302	0.667	0.5072	N	1, 60	6.101	0.0164
P	1.212	0.276	4.385	<0.0001	P	1, 60	24.497	<0.0001
GS	-1.884	0.295	-6.378	<0.0001	GS	1, 180	111.167	<0.0001
ploidy	0.624	0.295	2.111	0.0361	ploidy	1, 180	361.882	<0.0001
N : P	-0.506	0.381	-1.33	0.1885	N : P	1, 60	1.084	0.3019
N : GS	-0.368	0.427	-0.861	0.3904	N : GS	1, 180	11.836	0.0007
P : GS	-1.497	0.391	-3.83	0.0002	P : GS	1, 180	7.649	0.0063
N : ploidy	0.138	0.427	0.323	0.7468	N : ploidy	1, 180	18.713	<0.0001
P : ploidy	-1.364	0.391	-3.491	0.0006	P : ploidy	1, 180	4.092	0.0446
GS : ploidy	1.529	0.418	3.659	0.0003	GS : ploidy	1, 180	476.754	<0.0001
N : P : GS	0.311	0.538	0.577	0.5648	N : P : GS	1, 180	18.067	<0.0001
N : P : ploidy	-0.209	0.538	-0.388	0.6983	N : P : ploidy	1, 180	8.328	0.0044
N : GS : ploidy	-0.177	0.604	-0.292	0.7703	N : GS : ploidy	1, 180	27.93	<0.0001
P : GS : ploidy	2.34	0.553	4.234	<0.0001	P : GS : ploidy	1, 180	95.669	<0.0001
N : P : GS : ploidy	2.615	0.761	3.435	0.0007	N : P : GS : ploidy	1, 180	11.798	0.0007

Table S2. 5 continued

(b) Species number

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	4.356	0.098	44.548	<0.0001	Intercept	1, 180	6513.509	<0.0001
N	-0.478	0.141	-3.379	0.0013	N	1, 60	77.91	<0.0001
P	-0.458	0.129	-3.545	0.0008	P	1, 60	44.253	<0.0001
GS	-2.941	0.138	-21.272	<0.0001	GS	1, 180	1719.748	<0.0001
ploidy	-1.01	0.138	-7.301	<0.0001	ploidy	1, 180	28.194	<0.0001
N : P	-0.326	0.178	-1.829	0.0723	N : P	1, 60	5.2	0.0262
N : GS	0.365	0.2	1.824	0.0242	N : GS	1, 180	46.525	<0.0001
P : GS	0.2	0.183	1.091	0.1758	P : GS	1, 180	33.378	<0.0001
N : ploidy	0.007	0.2	0.035	0.9649	N : ploidy	1, 180	0.258	0.6118
P : ploidy	0.027	0.183	0.149	0.853	P : ploidy	1, 180	7.81	0.0058
GS : ploidy	2.142	0.196	10.951	<0.0001	GS : ploidy	1, 180	1227.501	<0.0001
N : P : GS	0.02	0.252	0.08	0.9207	N : P : GS	1, 180	0.763	0.3836
N : P : ploidy	-0.057	0.252	-0.226	0.7783	N : P : ploidy	1, 180	0.111	0.739
N : GS : ploidy	-0.054	0.283	-0.19	0.8133	N : GS : ploidy	1, 180	0.873	0.3515
P : GS : ploidy	0.294	0.259	1.138	0.1581	P : GS : ploidy	1, 180	8.032	0.0051
N : P : GS : ploidy	0.21	0.356	0.588	0.4649	N : P : GS : ploidy	1, 180	0.536	0.4649

(c) C-S-R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	4.897	0.351	13.941	<0.0001	Intercept	1, 180	4311.457	<0.0001
N	0.35	0.508	0.69	0.493	N	1, 60	4.514	0.0377
P	2.103	0.465	4.525	<0.0001	P	1, 60	22.648	<0.0001
GS	-3.34	0.497	-6.724	<0.0001	GS	1, 180	127.655	<0.0001

Table S2. 5c continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
ploidy	1.083	0.497	2.18	0.0305	ploidy	1, 180	371.2	<0.0001
N : P	-0.923	0.64	-1.442	0.1544	N : P	1, 60	0.613	0.4367
N : GS	-0.624	0.718	-0.868	0.3863	N : GS	1, 180	11.467	0.0009
P : GS	-2.574	0.657	-3.917	0.0001	P : GS	1, 180	7.245	0.0078
N : ploidy	0.23	0.718	0.32	0.7493	N : ploidy	1, 180	16.275	0.0001
P : ploidy	-2.374	0.657	-3.613	0.0004	P : ploidy	1, 180	2.899	0.0903
GS : ploidy	2.732	0.703	3.888	0.0001	GS : ploidy	1, 180	495.404	<0.0001
N : P : GS	0.604	0.905	0.667	0.5057	N : P : GS	1, 180	18.09	<0.0001
N : P : ploidy	-0.375	0.905	-0.414	0.6793	N : P : ploidy	1, 180	7.423	0.0071
N : GS : ploidy	-0.316	1.016	-0.311	0.7562	N : GS : ploidy	1, 180	25.867	<0.0001
P : GS : ploidy	3.999	0.929	4.303	<0.0001	P : GS : ploidy	1, 180	95.052	<0.0001
N : P : GS : ploidy	4.238	1.28	3.31	0.0011	N : P : GS : ploidy	1, 180	10.957	0.0011

(d) C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.543	0.158	9.785	<0.0001	Intercept	1, 180	2306.12	<0.0001
N	0.214	0.228	0.94	0.3508	N	1, 60	24.796	<0.0001
P	0.789	0.209	3.781	0.0004	P	1, 60	38.771	<0.0001
GS	-1.197	0.223	-5.37	<0.0001	GS	1, 180	58.754	<0.0001
ploidy	0.464	0.223	2.082	0.0313	ploidy	1, 180	411.583	<0.0001
N : P	-0.204	0.287	-0.711	0.4801	N : P	1, 60	3.489	0.0667
N : GS	-0.231	0.322	-0.716	0.4561	N : GS	1, 180	14.344	0.0002
P : GS	-0.867	0.295	-2.937	0.0025	P : GS	1, 180	13.775	0.0003
N : ploidy	0.152	0.322	0.47	0.6247	N : ploidy	1, 180	28.87	<0.0001

Table S2. 5d continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
P : ploidy	-0.778	0.295	-2.636	0.0066	P : ploidy	1, 180	11.163	0.001
GS : ploidy	0.965	0.315	3.061	0.0017	GS : ploidy	1, 180	425.739	<0.0001
N : P : GS	0.076	0.406	0.187	0.8453	N : P : GS	1, 180	18.824	<0.0001
N : P : ploidy	-0.189	0.406	-0.465	0.6282	N : P : ploidy	1, 180	11.399	0.0009
N : GS : ploidy	-0.169	0.456	-0.371	0.699	N : GS : ploidy	1, 180	35.335	<0.0001
P : GS : ploidy	1.494	0.417	3.582	0.0003	P : GS : ploidy	1, 180	94.449	<0.0001
N : P : GS : ploidy	2.239	0.575	3.895	0.0001	N : P : GS : ploidy	1, 180	16.502	0.0001

(e) S

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.753	0.113	15.552	<0.0001	Intercept	1, 180	4286.365	<0.0001
N	-0.07	0.163	-0.428	0.67	N	1, 60	3.18	0.0796
P	0.537	0.149	3.602	0.0006	P	1, 60	2.458	0.1222
GS	-0.998	0.159	-6.259	<0.0001	GS	1, 180	118.499	<0.0001
ploidy	0.314	0.159	1.967	0.0507	ploidy	1, 180	261.774	<0.0001
N : P	-0.34	0.205	-1.656	0.1029	N : P	1, 60	0.065	0.7998
N : GS	-0.054	0.231	-0.235	0.8146	N : GS	1, 180	11.517	0.0008
P : GS	-0.754	0.211	-3.577	0.0004	P : GS	1, 180	4.303	0.0395
N : ploidy	0.104	0.231	0.451	0.6526	N : ploidy	1, 180	11.367	0.0009
P : ploidy	-0.735	0.211	-3.486	0.0006	P : ploidy	1, 180	1.121	0.2912
GS : ploidy	0.73	0.225	3.236	0.0014	GS : ploidy	1, 180	349.74	<0.0001
N : P : GS	0.172	0.291	0.593	0.5539	N : P : GS	1, 180	11.28	0.001
N : P : ploidy	-0.062	0.291	-0.213	0.8315	N : P : ploidy	1, 180	4.922	0.0278
N : GS : ploidy	-0.145	0.326	-0.445	0.657	N : GS : ploidy	1, 180	13.477	0.0003

Table S2. 5e continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
P : GS : ploidy	1.207	0.298	4.047	0.0001	P : GS : ploidy	1, 180	73.004	<0.0001
N : P : GS : ploidy	1.035	0.411	2.52	0.0126	N : P : GS : ploidy	1, 180	6.349	0.0126

(f) R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.601	0.115	13.887	<0.0001	Intercept	1, 180	3952.054	<0.0001
N	0.206	0.167	1.234	0.222	N	1, 60	0.395	0.5321
P	0.777	0.153	5.094	<0.0001	P	1, 60	12.097	0.0009
GS	-1.145	0.163	-7.022	<0.0001	GS	1, 180	188.478	<0.0001
ploidy	0.305	0.163	1.873	0.0627	ploidy	1, 180	264.955	<0.0001
N : P	-0.379	0.21	-1.804	0.0763	N : P	1, 60	0.045	0.8329
N : GS	-0.339	0.236	-1.437	0.1525	N : GS	1, 180	4.131	0.0436
P : GS	-0.953	0.216	-4.418	<0.0001	P : GS	1, 180	1.706	0.1932
N : ploidy	-0.026	0.236	-0.108	0.9138	N : ploidy	1, 180	3.799	0.0529
P : ploidy	-0.861	0.216	-3.994	0.0001	P : ploidy	1, 180	0.052	0.8194
GS : ploidy	1.037	0.231	4.496	<0.0001	GS : ploidy	1, 180	504.959	<0.0001
N : P : GS	0.355	0.297	1.196	0.2333	N : P : GS	1, 180	15.884	0.0001
N : P : ploidy	-0.124	0.297	-0.417	0.677	N : P : ploidy	1, 180	2.904	0.0901
N : GS : ploidy	-0.002	0.333	-0.005	0.9962	N : GS : ploidy	1, 180	16.904	0.0001
P : GS : ploidy	1.298	0.305	4.254	<0.0001	P : GS : ploidy	1, 180	74.063	<0.0001
N : P : GS : ploidy	0.964	0.42	2.294	0.0229	N : P : GS : ploidy	1, 180	5.264	0.0229

Table S2. 6 ANOVA output testing the effect of N and P treatment on PGLS mean GS of each subplot: (**a**) phylogenetic least squares biomass–weighted mean GS (= 'biomass-weighted mean $1C_{pgls}$-value') which takes into account each taxon's contribution to total biomass; and (**b**) phylogeny-adjusted 'presence-absence mean $1C_{pgls}$ -value', where biomass was not taken into account. The intercepts below show the estimated PGLS mean subplot GS without N or P treatment (control plots), and the estimated coefficients in the second column show the effects of N application (i.e. without P), the effects of P application (i.e. without N), and the effects when both are applied on a subplot. The reference level is no application of N or P. Significant parameters are in bold (p-value < 0.05).

(a) Biomass-weighted mean 1C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA:	DF	F-value	p-value
Intercept	3.192	0.296	10.785	<0.00001	Intercept	1, 60	885.1916	<0.0001
N	0.052	0.428	0.121	0.90373	**N**	**1, 60**	**36.0665**	**<0.0001**
P	-0.504	0.397	-1.269	0.20940	**P**	**1, 60**	**6.7855**	**0.0116**
N : P	**2.250**	**0.542**	**4.151**	**0.00011**	**N : P**	**1, 60**	**17.2289**	**0.0001**

(b) Presence-absence mean 1C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA:	DF	F-value	p-value
Intercept	2.136	0.114	18.743	<0.00001	Intercept	1, 60	2436.64	<0.0001
N	0.001	0.165	0.003	0.99750	**N**	**1, 60**	**20.5973**	**<0.0001**
P	0.068	0.153	0.444	0.65850	**P**	**1, 60**	**15.2558**	**0.0002**
N : P	**0.630**	**0.209**	**3.021**	**0.00370**	**N : P**	**1, 60**	**9.1268**	**0.0037**

Table S2. 7 Different 1C-value thresholds for small vs large GS were investigated. This table shows means, ratios, and standard deviations where large GS ≥ 2.5 pg between the four genomic groups for: (**a**) total subplot biomass; (**b**) total number of species per subplot, and (**c**) total C-weighted; (**d**) total S-weighted; (**e**) total R-weighted biomass. As above, the four genomic groups of taxa are: i) diploid taxa with small GS ii) diploid taxa with big GS, iii) polyploid taxa with small GS; and iv) polyploid taxa with big GS. 1C= 1C-value (pg). The number of subplots per treatment is as follows: control=12, N=11, P=16, N+P= 25. See also Figs. S2.7 and S2.10.

(a)	**Mean total biomass (g)**				**Mean ratio total biomass**			
Treatment	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg
Control	3.293 ±2.09	6.63 ±3.77	3.959 ±1.96	17.825 ±3.44	0.099 ±0.06	0.201 ±0.1	0.122 ±0.05	0.578 ±0.16
N	4.1 ±1.66	6.697 ±3.61	4.052 ±3.15	19.62 ±6.16	0.12 ±0.05	0.198 ±0.12	0.115 ±0.08	0.567 ±0.16
P	12.206 ±8.7	5.579 ±3.16	2.706 ±2.17	23.995 ±6.54	0.251 ±0.11	0.14 ±0.09	0.064 ±0.05	0.545 ±0.1
N+P	12.218 ±6.8	2.83 ±1.6	0.068 ±0.18	43.246 ±9.63	0.202 ±0.11	0.051 ±0.03	0.001 ±0	0.746 ±0.11

(b)	**Mean total number of species**				**Mean ratio species number**			
Treatment	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg
Control	12 ±2	9 ±1	39 ±4	6 ±1	0.307 ±0.03	0.234 ±0.02	0.167 ±0.02	0.291 ±0.03
N	9 ±2	8 ±2	31 ±5	3 ±1	0.291 ±0.04	0.251 ±0.02	0.107 ±0.03	0.352 ±0.07
P	10 ±1	7 ±2	32 ±4	4 ±2	0.304 ±0.04	0.215 ±0.03	0.113 ±0.04	0.368 ±0.03
N+P	7 ±2	4 ±2	20 ±5	0 ±1	0.333 ±0.06	0.184 ±0.06	0.018 ±0.04	0.465 ±0.06

Table S2. 7 continued

(c)	C-weighted mean total biomass (g)				C-weighted mean biomass ratio			
Treatment	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg
Control	0.957 ±0.67	1.843 ±1.07	1.097 ±0.53	6.157 ±1.05	0.029 ±0.02	0.056 ±0.03	0.034 ±0.02	0.199 ±0.05
N	1.331 ±0.57	2.157 ±1.1	1.328 ±1.06	7.646 ±2.44	0.039 ±0.02	0.063 ±0.04	0.038 ±0.03	0.219 ±0.05
P	4.135 ±3.36	1.776 ±0.95	0.846 ±0.71	9.809 ±3.88	0.083 ±0.05	0.044 ±0.03	0.02 ±0.01	0.215 ±0.04
N+P	5.018 ±2.95	0.934 ±0.53	0.025 ±0.07	23.657 ±9.63	0.081 ±0.04	0.017 ±0.01	0.001 ±0	0.391 ±0.1

(d)	S-weighted mean total biomass (g)				S-weighted mean biomass ratio			
Treatment	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg
Control	1.224 ±0.69	2.825 ±1.62	1.764 ±1	5.816 ±1.21	0.037 ±0.02	0.085 ±0.04	0.054 ±0.03	0.189 ±0.06
N	1.287 ±0.53	2.37 ±1.54	1.407 ±1.07	5.96 ±1.93	0.038 ±0.02	0.071 ±0.05	0.04 ±0.03	0.173 ±0.05
P	3.785 ±2.2	2.009 ±1.26	1.025 ±0.76	7.066 ±1.8	0.08 ±0.03	0.051 ±0.04	0.025 ±0.02	0.164 ±0.04
N + P	2.893 ±1.79	0.961 ±0.55	0.025 ±0.07	9.733 ±1.83	0.05 ±0.03	0.017 ±0.01	0.001 ±0	0.176 ±0.06

(e)	R-weighted mean total biomass (g)				R-weighted mean biomass ratio			
Treatment	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg	Diploid 1C< 2.5pg	Diploid 1C≥ 2.5pg	Polyploid 1C< 2.5pg	Polyploid 1C≥ 2.5pg
Control	1.112 ±0.73	1.962 ±1.1	1.098 ±0.54	5.85 ±1.19	0.033 ±0.02	0.06 ±0.03	0.034 ±0.02	0.19 ±0.06
N	1.482 ±0.62	2.171 ±1.1	1.317 ±1.03	6.013 ±1.97	0.043 ±0.02	0.064 ±0.04	0.037 ±0.03	0.174 ±0.05
P	4.286 ±3.17	1.793 ±0.97	0.835 ±0.72	7.119 ±1.78	0.088 ±0.04	0.045 ±0.03	0.02 ±0.01	0.166 ±0.04
N+P	4.307 ±2.6	0.935 ±0.53	0.018 ±0.04	9.857 ±1.85	0.071 ±0.04	0.017 ±0.01	0 ±0	0.178 ±0.06

Table S2. 8 Large GS ≥ 2.5 pg. Treatment contrasts and ANOVA output testing the effects and interactions of N, P, GS (small vs large, where large GS ≥ 2.5 pg), and ploidy (diploid vs polyploid), on subplot: (**a**) total biomass; (**b**) total species numbers; (**d**) C-weighted biomass; (**e**) S-weighted biomass; and (f) R-weighted biomass testing for significance in treatment, GS, and ploidy. Part (**c**) shows multivariate ANOVA output where each C-S-R-weighted biomass are the response variables (i.e. n=3). Baseline levels in the contrasts are: without N, without P, diploid, and small GS for N, P, GS and ploidy effects respectively. Dependent variables (i.e. total biomass, species numbers, C, S, R-weighted biomass) were square-root transformed.

(a) Biomass

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.727	0.209	8.283	<0.0001	Intercept	1, 180	3903.145	<.0001
N	0.237	0.302	0.787	0.4345	N	1, 60	0.039	0.8436
P	1.588	0.276	5.757	<0.0001	P	1, 60	10.182	0.0023
GS	0.724	0.295	2.456	0.015	GS	1, 180	371.789	<.0001
ploidy	0.202	0.295	0.685	0.4941	ploidy	1, 180	74.589	<.0001
N : P	-0.232	0.380	-0.612	0.543	N : P	1, 60	1.089	0.3009
N : GS	-0.210	0.426	-0.492	0.6232	N : GS	1, 180	18.915	<.0001
P : GS	-1.780	0.390	-4.562	<0.0001	P : GS	1, 180	2.214	0.1385
N : ploidy	-0.258	0.426	-0.605	0.5459	N : ploidy	1, 180	1.756	0.1868
P : ploidy	-2.006	0.390	-5.143	<0.0001	P : ploidy	1, 180	2.570	0.1106
GS : ploidy	1.549	0.417	3.715	0.0003	GS : ploidy	1, 180	738.763	<.0001
N : P : GS	-0.459	0.537	-0.854	0.3944	N : P : GS	1, 180	10.145	0.0017
N : P : ploidy	-1.142	0.537	-2.125	0.0349	N : P : ploidy	1, 180	1.924	0.1671
N : GS : ploidy	0.409	0.603	0.679	0.4981	N : GS : ploidy	1, 180	71.494	<.0001
P : GS : ploidy	2.849	0.552	5.163	<0.0001	P : GS : ploidy	1, 180	147.459	<.0001
N : P : GS : ploidy	3.339	0.760	4.392	<0.0001	N : P : GS : ploidy	1, 180	19.294	<.0001

Table S2.8 continued

(b) Species number

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	3.450	0.107	32.112	<0.0001	Intercept	1, 180	4776.047	<.0001
N	-0.449	0.155	-2.889	0.0054	N	1, 60	93.639	<.0001
P	-0.343	0.142	-2.413	0.0189	P	1, 60	52.885	<.0001
GS	-0.442	0.110	-4.006	0.0001	GS	1, 180	385.281	<.0001
ploidy	-0.906	0.110	-8.222	<0.0001	ploidy	1, 180	126.444	<.0001
N : P	-0.093	0.196	-0.475	0.6363	N : P	1, 60	7.586	0.0078
N : GS	0.221	0.159	1.390	0.1664	N : GS	1, 180	55.622	<.0001
P : GS	-0.047	0.146	-0.322	0.7481	P : GS	1, 180	27.087	<.0001
N : ploidy	-0.279	0.159	-1.748	0.0821	N : ploidy	1, 180	12.714	0.0005
P : ploidy	-0.319	0.146	-2.187	0.03	P : ploidy	1, 180	0.898	0.3446
GS : ploidy	1.248	0.156	8.003	<0.0001	GS : ploidy	1, 180	1188.565	<.0001
N : P : GS	-0.394	0.201	-1.963	0.0512	N : P : GS	1, 180	0.140	0.7089
N : P : ploidy	-0.706	0.201	-3.514	0.0006	N : P : ploidy	1, 180	3.313	0.0704
N : GS : ploidy	0.414	0.225	1.837	0.0678	N : GS : ploidy	1, 180	69.089	<.0001
P : GS : ploidy	0.776	0.206	3.763	0.0002	P : GS : ploidy	1, 180	77.362	<.0001
N : P : GS : ploidy	0.895	0.284	3.150	0.0019	N : P : GS : ploidy	1, 180	9.921	0.0019

(c) C-S-R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	2.984	0.353	8.446	<0.0001	Intercept	1, 180	4000.850	<.0001
N	0.412	0.511	0.806	0.4236	N	1, 60	0.027	0.8708
P	2.752	0.467	5.888	<0.0001	P	1, 60	8.830	0.0043
GS	1.238	0.500	2.479	0.0141	GS	1, 180	374.461	<.0001

Table S2.8c continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
ploidy	0.328	0.500	0.656	0.5127	ploidy	1, 180	71.691	<.0001
N : P	-0.464	0.644	-0.721	0.4735	N : P	1, 60	1.801	0.1846
N : GS	-0.356	0.722	-0.493	0.6225	N : GS	1, 180	17.357	<.0001
P : GS	-3.064	0.661	-4.636	<0.0001	P : GS	1, 180	1.678	0.1969
N : ploidy	-0.420	0.722	-0.581	0.5621	N : ploidy	1, 180	1.220	0.2708
P : ploidy	-3.454	0.661	-5.227	<0.0001	P : ploidy	1, 180	3.556	0.061
GS : ploidy	2.729	0.707	3.862	0.0002	GS : ploidy	1, 180	744.348	<.0001
N : P : GS	-0.739	0.910	-0.812	0.4181	N : P : GS	1, 180	9.677	0.0022
N : P : ploidy	-1.938	0.910	-2.129	0.0347	N : P : ploidy	1, 180	1.558	0.2136
N : GS : ploidy	0.658	1.022	0.644	0.5206	N : GS : ploidy	1, 180	67.367	<.0001
P : GS : ploidy	4.850	0.935	5.189	<0.0001	P : GS : ploidy	1, 180	144.930	<.0001
N : P : GS : ploidy	5.483	1.288	4.258	<0.0001	N : P : GS : ploidy	1, 180	18.135	<.0001

(d) C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	0.919	0.156	5.881	<0.0001	Intercept	1, 180	2216.498	<.0001
N	0.193	0.226	0.855	0.396	N	1, 60	9.008	0.0039
P	0.981	0.207	4.745	<0.0001	P	1, 60	21.906	<.0001
GS	0.367	0.213	1.719	0.0874	GS	1, 180	351.150	<.0001
ploidy	0.097	0.213	0.453	0.6514	ploidy	1, 180	107.827	<.0001
N : P	0.014	0.285	0.048	0.9617	N : P	1, 60	0.030	0.8641
N : GS	-0.069	0.308	-0.225	0.8224	N : GS	1, 180	25.348	<.0001
P : GS	-0.987	0.282	-3.498	0.0006	P : GS	1, 180	6.941	0.0092
N : ploidy	-0.120	0.308	-0.389	0.6981	N : ploidy	1, 180	6.730	0.0103

Table S2.8d continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
P : ploidy	-1.153	0.282	-4.087	0.0001	P : ploidy	1, 180	0.002	0.9682
GS : ploidy	1.090	0.302	3.614	0.0004	GS : ploidy	1, 180	697.041	<.0001
N : P : GS	-0.501	0.389	-1.288	0.1993	N : P : GS	1, 180	10.430	0.0015
N : P : ploidy	-0.861	0.389	-2.216	0.028	N : P : ploidy	1, 180	3.678	0.0567
N : GS : ploidy	0.258	0.436	0.591	0.5556	N : GS : ploidy	1, 180	83.273	<.0001
P : GS : ploidy	1.762	0.399	4.415	<0.0001	P : GS : ploidy	1, 180	138.086	<.0001
N : P : GS : ploidy	2.777	0.550	5.052	<0.0001	N : P : GS : ploidy	1, 180	25.519	<.0001

(e) S

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.065	0.113	9.406	<0.0001	Intercept	1, 180	3958.037	<.0001
N	0.039	0.164	0.239	0.812	N	1, 60	15.067	0.0003
P	0.811	0.150	5.417	<0.0001	P	1, 60	0.002	0.9659
GS	0.539	0.160	3.364	0.0009	GS	1, 180	338.458	<.0001
ploidy	0.215	0.160	1.345	0.1802	ploidy	1, 180	49.929	<.0001
N : P	-0.301	0.206	-1.460	0.1494	N : P	1, 60	3.961	0.0511
N : GS	-0.192	0.232	-0.828	0.4089	N : GS	1, 180	14.325	0.0002
P : GS	-1.069	0.212	-5.048	<0.0001	P : GS	1, 180	0.539	0.4637
N : ploidy	-0.191	0.232	-0.827	0.4093	N : ploidy	1, 180	0.311	0.5778
P : ploidy	-1.162	0.212	-5.485	<0.0001	P : ploidy	1, 180	5.911	0.016
GS : ploidy	0.580	0.226	2.562	0.0112	GS : ploidy	1, 180	542.580	<.0001
N : P : GS	0.036	0.292	0.124	0.9015	N : P : GS	1, 180	8.425	0.0042
N : P : ploidy	-0.407	0.292	-1.395	0.1648	N : P : ploidy	1, 180	0.570	0.4512
N : GS : ploidy	0.359	0.327	1.095	0.2749	N : GS : ploidy	1, 180	46.252	<.0001

Table S2.8e continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
P : GS : ploidy	1.659	0.300	5.537	<0.0001	P : GS : ploidy	1, 180	119.450	<.0001
N : P : GS : ploidy	1.126	0.413	2.727	0.007	N : P : GS : ploidy	1, 180	7.439	0.007

(f) R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.000	0.119	8.422	<0.0001	Intercept	1, 180	3613.775	<.0001
N	0.179	0.172	1.044	0.3005	N	1, 60	1.269	0.2643
P	0.959	0.157	6.110	<0.0001	P	1, 60	4.121	0.0468
GS	0.333	0.168	1.985	0.0486	GS	1, 180	263.553	<.0001
ploidy	0.016	0.168	0.094	0.9253	ploidy	1, 180	27.726	<.0001
N : P	-0.177	0.216	-0.818	0.4169	N : P	1, 60	5.499	0.0223
N : GS	-0.095	0.243	-0.393	0.6951	N : GS	1, 180	5.722	0.0178
P : GS	-1.007	0.222	-4.537	<0.0001	P : GS	1, 180	0.037	0.8472
N : ploidy	-0.108	0.243	-0.446	0.6561	N : ploidy	1, 180	0.292	0.5894
P : ploidy	-1.139	0.222	-5.131	<0.0001	P : ploidy	1, 180	11.182	0.001
GS : ploidy	1.058	0.237	4.457	<0.0001	GS : ploidy	1, 180	646.927	<.0001
N : P : GS	-0.274	0.306	-0.897	0.3712	N : P : GS	1, 180	5.693	0.0181
N : P : ploidy	-0.670	0.306	-2.189	0.0299	N : P : ploidy	1, 180	0.311	0.5776
N : GS : ploidy	0.041	0.343	0.121	0.9041	N : GS : ploidy	1, 180	40.272	<.0001
P : GS : ploidy	1.429	0.314	4.551	<0.0001	P : GS : ploidy	1, 180	109.699	<.0001
N : P : GS : ploidy	1.581	0.433	3.654	0.0003	N : P : GS : ploidy	1, 180	13.352	0.0003

Table S2. 9 Large GS ≥ 3 pg. Means, ratios, and standard deviations per nutrient treatment for: (**a**) total subplot biomass; (**b**) total number of species per subplot, and (**c**) total C-weighted; (**d**) total S-weighted; **(e**) total R-weighted biomass for the four genomic groupings. See also figures S2.8 and S2.11

(a)	**Mean total biomass (g)**				**Mean ratio total biomass**			
Treatment	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg
Control	7.357 ±4.2	2.566 ±1.63	4.153 ±2.13	17.631 ±3.55	0.223 ±0.11	0.077 ±0.04	0.127 ±0.06	0.572 ±0.17
N	8.205 ±4.79	2.593 ±1.33	4.199 ±3.09	19.473 ±6.2	0.243 ±0.15	0.075 ±0.04	0.119 ±0.08	0.562 ±0.16
P	14.651 ±7.76	3.134 ±1.78	3.025 ±2.2	23.677 ±6.74	0.317 ±0.09	0.074 ±0.05	0.072 ±0.05	0.537 ±0.11
N+P	12.452 ±6.96	2.596 ±1.53	0.13 ±0.22	43.184 ±9.66	0.206 ±0.11	0.047 ±0.03	0.003 ±0	0.745 ±0.11

(b)	**Mean total number of species**				**Mean ratio species number**			
Treatment	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg
Control	14 ±2	8 ±1	8 ±1	10 ±1	0.345 ±0.03	0.196 ±0.03	0.208 ±0.02	0.251 ±0.03
N	11 ±3	6 ±1	5 ±2	9 ±1	0.34 ±0.04	0.201 ±0.03	0.159 ±0.04	0.3 ±0.07
P	11 ±1	6 ±1	6 ±2	10 ±1	0.348 ±0.04	0.171 ±0.03	0.168 ±0.05	0.313 ±0.03
N+P	7 ±2	3 ±1	6 ±2	8 ±2	0.358 ±0.06	0.159 ±0.05	0.058 ±0.06	0.425 ±0.06

(c)	**C-weighted mean total biomass (g)**				**C-weighted mean biomass ratio**			
Treatment	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg
Control	2.323 ±1.39	0.477 ±0.33	1.15 ±0.57	6.104 ±1.08	0.07 ±0.04	0.014 ±0.01	0.036 ±0.02	0.197 ±0.05
N	2.737 ±1.61	0.751 ±0.45	1.366 ±1.04	7.609 ±2.46	0.081 ±0.05	0.021 ±0.01	0.039 ±0.03	0.218 ±0.05
P	4.975 ±3.08	0.936 ±0.5	0.943 ±0.71	9.712 ±3.93	0.106 ±0.04	0.022 ±0.01	0.022 ±0.01	0.212 ±0.04
N+P	5.115 ±3.01	0.836 ±0.5	0.045 ±0.08	23.638 ±9.64	0.083 ±0.04	0.015 ±0.01	0.001 ±0	0.391 ±0.1

Table S2.9 continued

(d)	S-weighted mean total biomass (g)				S-weighted mean biomass ratio			
Treatment	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg
Control	2.556 ±1.38	1.493 ±0.95	1.817 ±1.04	5.763 ±1.24	0.078 ±0.04	0.045 ±0.03	0.055 ±0.03	0.187 ±0.06
N	2.58 ±1.59	1.076 ±0.77	1.444 ±1.06	5.922 ±1.95	0.077 ±0.05	0.032 ±0.02	0.041 ±0.03	0.172 ±0.05
P	4.549 ±1.83	1.245 ±0.79	1.122 ±0.78	6.969 ±1.87	0.101 ±0.02	0.03 ±0.02	0.027 ±0.02	0.162 ±0.05
N + P	2.932 ±1.83	0.922 ±0.54	0.045 ±0.08	9.713 ±1.84	0.05 ±0.03	0.017 ±0.01	0.001 ±0	0.176 ±0.06

(e)	R-weighted mean total biomass (g)				R-weighted mean biomass ratio			
Treatment	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg	Diploid 1C< 3pg	Diploid 1C≥ 3pg	Polyploid 1C< 3pg	Polyploid 1C≥ 3pg
Control	2.477 ±1.44	0.596 ±0.36	1.185 ±0.6	5.763 ±1.24	0.075 ±0.04	0.018 ±0.01	0.036 ±0.02	0.187 ±0.06
N	2.887 ±1.62	0.765 ±0.44	1.388 ±0.99	5.941 ±1.98	0.085 ±0.05	0.022 ±0.01	0.039 ±0.02	0.172 ±0.05
P	5.126 ±2.88	0.953 ±0.51	0.959 ±0.72	6.994 ±1.86	0.11 ±0.03	0.022 ±0.01	0.023 ±0.02	0.163 ±0.04
N+P	4.404 ±2.68	0.838 ±0.5	0.04 ±0.06	9.835 ±1.86	0.073 ±0.04	0.015 ±0.01	0.001 ±0	0.178 ±0.06

Table S2. 10 Large GS ≥ 3 pg. Treatment contrasts and ANOVA output testing the effects and interactions of N, P, GS (small vs large, where large GS ≥ 3 pg), ploidy (diploid vs polyploid), on square-root transformed subplot dependent variables of: (**a**) total biomass; (**b**) total species numbers; (**d**) C-weighted biomass; (**e**) S-weighted biomass; and (**f**) R-weighted biomass testing for significance in treatment, GS, and ploidy. Part (**c**) shows multivariate ANOVA output where each C-S-R-weighted biomass are the response variables (i.e. n=3). Baseline levels in the contrasts are: without N, without P, diploid, and small GS for N, P, GS and ploidy effects respectively (e.g. there is a 0.2 g increase in biomass with the addition of N, relative to subplots without N).

(a) Biomass

	Estimate	Std. error	t value	Pr(>|t|)
Intercept	2.582	0.204	12.662	<0.0001
N	0.130	0.295	0.442	0.6597
P	1.140	0.270	4.227	0.0001
GS	-1.066	0.288	-3.695	0.0003
ploidy	-0.610	0.288	-2.115	0.0358
N : P	-0.504	0.372	-1.355	0.1804
N : GS	-0.091	0.417	-0.217	0.8283
P : GS	-0.967	0.381	-2.535	0.0121
N : ploidy	-0.148	0.417	-0.354	0.7237
P : ploidy	-1.487	0.381	-3.899	0.0001
GS : ploidy	3.273	0.408	8.025	<0.0001
N : P : GS	0.302	0.525	0.575	0.5662
N : P : ploidy	-0.876	0.525	-1.667	0.0973
N : GS : ploidy	0.293	0.590	0.497	0.62
P : GS : ploidy	1.952	0.539	3.619	0.0004
N : P : GS : ploidy	2.608	0.743	3.509	0.0006

ANOVA	DF	F-value	p-value
Intercept	1, 180	4059.450	<.0001
N	1, 60	0.180	0.6729
P	1, 60	12.523	0.0008
GS	1, 180	195.728	<.0001
ploidy	1, 180	96.050	<.0001
N : P	1, 60	0.556	0.4588
N : GS	1, 180	43.379	<.0001
P : GS	1, 180	21.261	<.0001
N : ploidy	1, 180	1.717	0.1917
P : ploidy	1, 180	2.370	0.1254
GS : ploidy	1, 180	1044.294	<.0001
N : P : GS	1, 180	18.678	<.0001
N : P : ploidy	1, 180	1.327	0.2508
N : GS : ploidy	1, 180	43.361	<.0001
P : GS : ploidy	1, 180	80.368	<.0001
N : P : GS : ploidy	1, 180	12.313	0.0006

Table S2. 10 continued

(b) Species number

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	3.661	0.113	32.256	<0.0001	Intercept	1, 180	4875.466	<.0001
N	-0.413	0.164	-2.518	0.0145	N	1, 60	78.190	<.0001
P	-0.339	0.150	-2.261	0.0274	P	1, 60	43.865	<.0001
GS	-0.913	0.124	-7.350	<0.0001	GS	1, 180	13.348	0.0003
ploidy	-0.825	0.124	-6.643	<0.0001	ploidy	1, 180	40.566	<.0001
N : P	-0.244	0.207	-1.179	0.2432	N : P	1, 60	6.066	0.0167
N : GS	0.156	0.180	0.868	0.3863	N : GS	1, 180	53.523	<.0001
P : GS	-0.069	0.164	-0.420	0.6747	P : GS	1, 180	25.397	<.0001
N : ploidy	-0.205	0.180	-1.140	0.2557	N : ploidy	1, 180	3.810	0.0525
P : ploidy	-0.190	0.164	-1.158	0.2483	P : ploidy	1, 180	0.118	0.7319
GS : ploidy	1.184	0.176	6.739	<0.0001	GS : ploidy	1, 180	741.941	<.0001
N : P : GS	-0.071	0.226	-0.312	0.7556	N : P : GS	1, 180	3.160	0.0772
N : P : ploidy	-0.553	0.226	-2.442	0.0156	N : P : ploidy	1, 180	1.525	0.2185
N : GS : ploidy	0.353	0.254	1.388	0.167	N : GS : ploidy	1, 180	36.376	<.0001
P : GS : ploidy	0.644	0.232	2.770	0.0062	P : GS : ploidy	1, 180	40.567	<.0001
N : P : GS : ploidy	0.710	0.320	2.218	0.0278	N : P : GS : ploidy	1, 180	4.921	0.0278

(c) C-S-R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	4.469	0.345	12.972	<0.0001	Intercept	1, 180	4176.040	<.0001
N	0.222	0.498	0.446	0.6572	N	1, 60	0.008	0.9299
P	1.971	0.456	4.326	0.0001	P	1, 60	11.461	0.0013
GS	-1.930	0.487	-3.961	0.0001	GS	1, 180	193.245	<.0001

Table S2. 10c continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
ploidy	-1.080	0.487	-2.217	0.0279	ploidy	1, 180	94.740	<.0001
N : P	-0.930	0.628	-1.482	0.1437	N : P	1, 60	1.150	0.2879
N : GS	-0.104	0.705	-0.148	0.8825	N : GS	1, 180	42.347	<.0001
P : GS	-1.592	0.645	-2.470	0.0145	P : GS	1, 180	20.809	<.0001
N : ploidy	-0.227	0.705	-0.322	0.7479	N : ploidy	1, 180	1.106	0.2943
P : ploidy	-2.552	0.645	-3.959	0.0001	P : ploidy	1, 180	3.572	0.0604
GS : ploidy	5.778	0.689	8.385	<0.0001	GS : ploidy	1, 180	1066.735	<.0001
N : P : GS	0.537	0.888	0.605	0.546	N : P : GS	1, 180	18.035	<.0001
N : P : ploidy	-1.479	0.888	-1.666	0.0975	N : P : ploidy	1, 180	1.073	0.3018
N : GS : ploidy	0.413	0.996	0.415	0.6789	N : GS : ploidy	1, 180	39.251	<.0001
P : GS : ploidy	3.234	0.912	3.547	0.0005	P : GS : ploidy	1, 180	76.339	<.0001
N : P : GS : ploidy	4.258	1.256	3.391	0.0009	N : P : GS : ploidy	1, 180	11.501	0.0009

(d) C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.442	0.154	9.357	<0.0001	Intercept	1, 180	2145.746	<.0001
N	0.118	0.223	0.528	0.5991	N	1, 60	10.156	0.0023
P	0.706	0.204	3.463	0.001	P	1, 60	24.380	<.0001
GS	-0.793	0.208	-3.815	0.0002	GS	1, 180	204.517	<.0001
ploidy	-0.403	0.208	-1.940	0.0539	ploidy	1, 180	134.705	<.0001
N : P	-0.138	0.281	-0.492	0.6247	N : P	1, 60	0.109	0.7421
N : GS	0.068	0.301	0.228	0.8202	N : GS	1, 180	49.820	<.0001
P : GS	-0.427	0.275	-1.552	0.1223	P : GS	1, 180	29.332	<.0001

Table S2. 10d continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
N : ploidy	-0.045	0.301	-0.150	0.8807	N : ploidy	1, 180	6.401	0.0123
P : ploidy	-0.837	0.275	-3.044	0.0027	P : ploidy	1, 180	0.000	0.9862
GS : ploidy	2.217	0.294	7.538	<0.0001	GS : ploidy	1, 180	968.394	<.0001
N : P : GS	-0.108	0.379	-0.286	0.7751	N : P : GS	1, 180	16.538	0.0001
N : P : ploidy	-0.709	0.379	-1.871	0.063	N : P : ploidy	1, 180	3.332	0.0696
N : GS : ploidy	0.124	0.425	0.293	0.7701	N : GS : ploidy	1, 180	56.565	<.0001
P : GS : ploidy	1.155	0.389	2.968	0.0034	P : GS : ploidy	1, 180	81.635	<.0001
N : P : GS : ploidy	2.396	0.536	4.471	<0.0001	N : P : GS : ploidy	1, 180	19.993	<.0001

(e) S

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.532	0.111	13.846	<0.0001	Intercept	1, 180	4157.026	<.0001
N	-0.011	0.160	-0.072	0.9432	N	1, 60	14.683	0.0003
P	0.565	0.146	3.863	0.0003	P	1, 60	0.028	0.8686
GS	-0.376	0.156	-2.401	0.0174	GS	1, 180	179.992	<.0001
ploidy	-0.234	0.156	-1.498	0.1358	ploidy	1, 180	62.095	<.0001
N : P	-0.462	0.202	-2.292	0.0254	N : P	1, 60	2.762	0.1017
N : GS	-0.167	0.226	-0.738	0.4612	N : GS	1, 180	34.986	<.0001
P : GS	-0.665	0.207	-3.212	0.0016	P : GS	1, 180	13.983	0.0002
N : ploidy	-0.139	0.226	-0.613	0.5406	N : ploidy	1, 180	0.415	0.5203
P : ploidy	-0.875	0.207	-4.228	<0.0001	P : ploidy	1, 180	4.860	0.0288
GS : ploidy	1.466	0.221	6.623	<0.0001	GS : ploidy	1, 180	789.182	<.0001
N : P : GS	0.493	0.285	1.728	0.0857	N : P : GS	1, 180	17.068	0.0001
N : P : ploidy	-0.244	0.285	-0.855	0.3934	N : P : ploidy	1, 180	0.228	0.6334

Table S2. 10e continued

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
N : GS : ploidy	0.335	0.320	1.047	0.2964	N : GS : ploidy	1, 180	24.492	<.0001
P : GS : ploidy	1.205	0.293	4.116	0.0001	P : GS : ploidy	1, 180	60.296	<.0001
N : P : GS : ploidy	0.681	0.403	1.688	0.0932	N : P : GS : ploidy	1, 180	2.848	0.0932

(f) R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
Intercept	1.495	0.116	12.884	<0.0001	Intercept	1, 180	3734.925	<.0001
N	0.116	0.168	0.690	0.4925	N	1, 60	0.722	0.399
P	0.700	0.154	4.561	<0.0001	P	1, 60	6.297	0.0148
GS	-0.761	0.164	-4.637	<0.0001	GS	1, 180	107.337	<.0001
ploidy	-0.442	0.164	-2.695	0.0077	ploidy	1, 180	44.613	<.0001
N : P	-0.330	0.212	-1.561	0.1238	N : P	1, 60	4.380	0.0406
N : GS	-0.006	0.237	-0.024	0.9811	N : GS	1, 180	22.444	<.0001
P : GS	-0.500	0.217	-2.303	0.0224	P : GS	1, 180	9.709	0.0021
N : ploidy	-0.043	0.237	-0.181	0.8568	N : ploidy	1, 180	0.486	0.4868
P : ploidy	-0.839	0.217	-3.866	0.0002	P : ploidy	1, 180	12.160	0.0006
GS : ploidy	2.095	0.232	9.027	<0.0001	GS : ploidy	1, 180	945.730	<.0001
N : P : GS	0.153	0.299	0.511	0.61	N : P : GS	1, 180	12.367	0.0006
N : P : ploidy	-0.526	0.299	-1.759	0.0803	N : P : ploidy	1, 180	0.094	0.7597
N : GS : ploidy	-0.046	0.336	-0.138	0.8902	N : GS : ploidy	1, 180	18.946	<.0001
P : GS : ploidy	0.874	0.307	2.847	0.0049	P : GS : ploidy	1, 180	50.246	<.0001
N : P : GS : ploidy	1.182	0.423	2.794	0.0058	N : P : GS : ploidy	1, 180	7.807	0.0058

Table S2. 11 Large GS ≥ 6 pg. Means, ratios, and standard deviations per nutrient treatment for: (**a**) total subplot biomass; (**b**) total number of species per subplot, and (**c**) total C-weighted; (**d**) total S-weighted; (**e**) total R-weighted biomass for the four genomic groupings. See also figures S2.9 and S2.12.

(a)	Mean total biomass (g)				Mean ratio total biomass			
Treatment	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg
Control	8.961 ±4.99	0.962 ±1.03	12.061 ±2.41	9.722 ±2.02	0.273 ±0.14	0.027 ±0.03	0.385 ±0.09	0.314 ±0.09
N	9.928 ±4.73	0.87 ±1.07	14.929 ±6.01	8.743 ±3.8	0.292 ±0.15	0.027 ±0.03	0.425 ±0.13	0.257 ±0.12
P	17.237 ±8.61	0.548 ±0.59	11.801 ±6.89	14.901 ±6.19	0.377 ±0.11	0.014 ±0.02	0.282 ±0.16	0.327 ±0.06
N+P	14.845 ±6.91	0.203 ±0.35	8.962 ±4.06	34.352 ±10.3	0.249 ±0.1	0.004 ±0.01	0.163 ±0.09	0.584 ±0.11

(b)	Mean total number of species				Mean ratio species number			
Treatment	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg
Control	20 ±3	1 ±0	11 ±2	6 ±1	0.516 ±0.04	0.026 ±0	0.29 ±0.03	0.168 ±0.02
N	16 ±4	1 ±0	8 ±2	6 ±1	0.503 ±0.05	0.039 ±0.01	0.268 ±0.02	0.191 ±0.05
P	16 ±2	1 ±0	9 ±2	7 ±0	0.486 ±0.03	0.034 ±0.01	0.266 ±0.04	0.215 ±0.02
N+P	10 ±3	1 ±1	4 ±1	5 ±2	0.48 ±0.05	0.038 ±0.03	0.218 ±0.05	0.265 ±0.04

(c)	C-weighted mean total biomass (g)				C-weighted mean biomass ratio			
Treatment	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg
Control	2.64 ±1.57	0.16 ±0.17	4.069 ±0.85	3.185 ±0.67	0.08 ±0.04	0.005 ±0	0.13 ±0.03	0.103 ±0.03
N	3.344 ±1.59	0.144 ±0.18	5.836 ±2.39	3.138 ±1.23	0.098 ±0.05	0.004 ±0.01	0.165 ±0.05	0.092 ±0.04
P	5.82 ±3.37	0.091 ±0.1	4.361 ±2.45	6.294 ±3.9	0.125 ±0.04	0.002 ±0	0.102 ±0.05	0.132 ±0.05
N+P	5.918 ±3.04	0.034 ±0.06	4.134 ±1.83	19.548 ±9.1	0.097 ±0.04	0.001 ±0	0.072 ±0.03	0.32 ±0.1

Table S2.11 continued

(d)	S-weighted mean total biomass (g)				S-weighted mean biomass ratio			
Treatment	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg
Control	3.408 ±1.78	0.641 ±0.68	4.312 ±0.86	3.268 ±0.68	0.104 ±0.05	0.018 ±0.02	0.137 ±0.03	0.106 ±0.03
N	3.077 ±1.62	0.579 ±0.72	4.574 ±1.86	2.793 ±1.29	0.091 ±0.05	0.018 ±0.02	0.131 ±0.04	0.082 ±0.04
P	5.43 ±2.12	0.365 ±0.39	3.801 ±2.23	4.291 ±1.27	0.121 ±0.03	0.009 ±0.01	0.092 ±0.05	0.097 ±0.02
N + P	3.718 ±1.72	0.136 ±0.23	2.416 ±1.41	7.342 ±1.59	0.065 ±0.03	0.003 ±0	0.046 ±0.03	0.131 ±0.04

(e)	R-weighted mean total biomass (g)				R-weighted mean biomass ratio			
Treatment	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg	Diploid 1C< 6pg	Diploid 1C≥ 6pg	Polyploid 1C< 6pg	Polyploid 1C≥ 6pg
Control	2.914 ±1.65	0.16 ±0.17	3.68 ±0.86	3.268 ±0.68	0.089 ±0.04	0.005 ±0	0.118 ±0.03	0.106 ±0.03
N	3.507 ±1.6	0.146 ±0.18	4.518 ±1.83	2.812 ±1.31	0.103 ±0.05	0.004 ±0.01	0.129 ±0.04	0.083 ±0.04
P	5.986 ±3.16	0.092 ±0.1	3.638 ±2.27	4.316 ±1.3	0.13 ±0.04	0.002 ±0	0.087 ±0.05	0.098 ±0.02
N+P	5.208 ±2.67	0.034 ±0.06	2.412 ±1.41	7.464 ±1.65	0.087 ±0.04	0.001 ±0	0.046 ±0.03	0.133 ±0.04

Table S2. 12 Large GS ≥ 6 pg. Treatment contrasts and ANOVA output testing the effects and interactions of N, P, GS (small vs large, where large GS ≥ 6 pg), ploidy (diploid vs polyploid), on subplot: (**a**) total biomass; (**b**) total species numbers; (**d**) C-weighted biomass; (**e**) S-weighted biomass; and (**f**) R-weighted biomass testing for significance in treatment, GS, and ploidy. Dependent variables were square-root transformed. Part (**c**) shows multivariate ANOVA output where each C-S-R-weighted biomass are the response variables (i.e. n=3). Baseline levels in the contrasts are: without N, without P, diploid, and small GS for N, P, GS and ploidy effects respectively (e.g. there is a 0.2 g increase in biomass with the addition of N, relative to subplots without N).

(a) Biomass

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	2.866	0.209	13.711	<0.0001	(Intercept)	1, 180	4150.617	<.0001
N	0.173	0.302	0.571	0.5699	N	1, 60	6.504	0.0133
P	1.181	0.277	4.270	0.0001	P	1, 60	25.505	<.0001
GS	-2.020	0.296	-6.833	<0.0001	GS	1, 180	115.179	<.0001
ploidy	0.591	0.296	1.998	0.0472	ploidy	1, 180	365.676	<.0001
N : P	-0.480	0.381	-1.259	0.2128	N : P	1, 60	0.996	0.3222
N : GS	-0.265	0.427	-0.619	0.5366	N : GS	1, 180	12.943	0.0004
P : GS	-1.379	0.391	-3.525	0.0005	P : GS	1, 180	8.740	0.0035
N : ploidy	0.167	0.427	0.390	0.6966	N : ploidy	1, 180	17.981	<.0001
P : ploidy	-1.334	0.391	-3.410	0.0008	P : ploidy	1, 180	3.674	0.0569
GS : ploidy	1.665	0.418	3.982	0.0001	GS : ploidy	1, 180	484.650	<.0001
N : P : GS	0.225	0.539	0.418	0.6766	N : P : GS	1, 180	17.100	0.0001
N : P : ploidy	-0.236	0.539	-0.437	0.6625	N : P : ploidy	1, 180	8.563	0.0039
N : GS : ploidy	-0.280	0.605	-0.463	0.6441	N : GS : ploidy	1, 180	26.225	<.0001
P : GS : ploidy	2.221	0.553	4.017	0.0001	P : GS : ploidy	1, 180	91.794	<.0001
N : P : GS : ploidy	2.700	0.762	3.544	0.0005	N : P : GS : ploidy	1, 180	12.563	0.0005

Table S2.12 continued

(b) Species number

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	4.470	0.097	46.096	<0.0001	(Intercept)	1, 180	6640.214	<.0001
N	-0.522	0.140	-3.724	0.0004	N	1, 60	71.515	<.0001
P	-0.534	0.128	-4.162	0.0001	P	1, 60	36.793	<.0001
GS	-3.470	0.111	-31.120	<0.0001	GS	1, 180	1944.982	<.0001
ploidy	-1.123	0.111	-10.077	<0.0001	ploidy	1, 180	46.763	<.0001
N : P	-0.320	0.177	-1.812	0.0751	N : P	1, 60	5.919	0.018
N : GS	0.597	0.161	3.706	0.0003	N : GS	1, 180	71.875	<.0001
P : GS	0.560	0.147	3.795	0.0002	P : GS	1, 180	64.411	<.0001
N : ploidy	0.051	0.161	0.319	0.7498	N : ploidy	1, 180	0.211	0.6468
P : ploidy	0.103	0.147	0.696	0.4874	P : ploidy	1, 180	2.303	0.1308
GS : ploidy	2.670	0.158	16.932	<0.0001	GS : ploidy	1, 180	1420.937	<.0001
N : P : GS	-0.045	0.203	-0.219	0.8267	N : P : GS	1, 180	0.415	0.5201
N : P : ploidy	-0.063	0.203	-0.310	0.7572	N : P : ploidy	1, 180	0.267	0.6061
N : GS : ploidy	-0.286	0.228	-1.256	0.2107	N : GS : ploidy	1, 180	0.571	0.4507
P : GS : ploidy	-0.066	0.209	-0.315	0.7529	P : GS : ploidy	1, 180	0.301	0.5837
N : P : GS : ploidy	0.274	0.287	0.954	0.3411	N : P : GS : ploidy	1, 180	0.911	0.3411

(c) C-S-R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	4.953	0.352	14.090	<0.0001	(Intercept)	1, 180	4290.323	<.0001
N	0.302	0.508	0.594	0.5545	N	1, 60	4.866	0.0312
P	2.051	0.465	4.411	<0.0001	P	1, 60	23.654	<.0001
GS	-3.571	0.497	-7.183	<0.0001	GS	1, 180	132.048	<.0001

Table S2.12c continued

ploidy	1.028	0.497	2.067	0.0402	ploidy	1, 180	375.223	<.0001
N : P	-0.879	0.641	-1.372	0.1751	N : P	1, 60	0.549	0.4614
N : GS	-0.452	0.719	-0.629	0.5303	N : GS	1, 180	12.562	0.0005
P : GS	-2.374	0.658	-3.610	0.0004	P : GS	1, 180	8.331	0.0044
N : ploidy	0.278	0.719	0.387	0.6993	N : ploidy	1, 180	15.594	0.0001
P : ploidy	-2.323	0.658	-3.532	0.0005	P : ploidy	1, 180	2.540	0.1127
GS : ploidy	2.962	0.703	4.214	<0.0001	GS : ploidy	1, 180	503.693	<.0001
N : P : GS	0.462	0.906	0.511	0.6103	N : P : GS	1, 180	17.141	0.0001
N : P : ploidy	-0.419	0.906	-0.463	0.6441	N : P : ploidy	1, 180	7.638	0.0063
N : GS : ploidy	-0.488	1.017	-0.480	0.6319	N : GS : ploidy	1, 180	24.229	<.0001
P : GS : ploidy	3.799	0.930	4.085	0.0001	P : GS : ploidy	1, 180	91.136	<.0001
N : P : GS : ploidy	4.379	1.281	3.418	0.0008	N : P : GS : ploidy	1, 180	11.684	0.0008

(d) C

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	1.543	0.158	9.780	<0.0001	(Intercept)	1, 180	2303.550	<.0001
N	0.217	0.228	0.950	0.346	N	1, 60	24.675	<.0001
P	0.789	0.209	3.780	0.0004	P	1, 60	39.104	<.0001
GS	-1.197	0.214	-5.596	<0.0001	GS	1, 180	59.277	<.0001
ploidy	0.464	0.214	2.169	0.0314	ploidy	1, 180	412.279	<.0001
N : P	-0.207	0.287	-0.719	0.4748	N : P	1, 60	3.626	0.0617
N : GS	-0.255	0.309	-0.824	0.411	N : GS	1, 180	14.184	0.0002
P : GS	-0.871	0.283	-3.076	0.0024	P : GS	1, 180	14.040	0.0002
N : ploidy	0.149	0.309	0.482	0.6302	N : ploidy	1, 180	28.980	<.0001
P : ploidy	-0.778	0.283	-2.748	0.0066	P : ploidy	1, 180	10.942	0.0011

Table S2.12d continued

GS : ploidy	0.965	0.303	3.190	0.0017	GS : ploidy	1, 180	426.734	<.0001
N : P : GS	0.105	0.390	0.268	0.7889	N : P : GS	1, 180	19.245	<.0001
N : P : ploidy	-0.186	0.390	-0.478	0.6332	N : P : ploidy	1, 180	11.100	0.001
N : GS : ploidy	-0.145	0.438	-0.332	0.7402	N : GS : ploidy	1, 180	35.499	<.0001
P : GS : ploidy	1.499	0.400	3.743	0.0002	P : GS : ploidy	1, 180	93.559	<.0001
N : P : GS : ploidy	2.210	0.551	4.008	0.0001	N : P : GS : ploidy	1, 180	16.061	0.0001

(e) S

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	1.779	0.113	15.761	<0.0001	(Intercept)	1, 180	4258.283	<.0001
N	-0.093	0.163	-0.568	0.5722	N	1, 60	2.876	0.0951
P	0.513	0.149	3.433	0.0011	P	1, 60	2.783	0.1005
GS	-1.088	0.160	-6.818	<0.0001	GS	1, 180	123.331	<.0001
ploidy	0.287	0.160	1.799	0.0737	ploidy	1, 180	264.659	<.0001
N : P	-0.319	0.206	-1.551	0.1261	N : P	1, 60	0.089	0.7663
N : GS	0.017	0.231	0.075	0.9405	N : GS	1, 180	12.874	0.0004
P : GS	-0.675	0.211	-3.195	0.0017	P : GS	1, 180	5.309	0.0224
N : ploidy	0.127	0.231	0.550	0.5833	N : ploidy	1, 180	10.769	0.0012
P : ploidy	-0.711	0.211	-3.365	0.0009	P : ploidy	1, 180	0.910	0.3414
GS : ploidy	0.820	0.226	3.633	0.0004	GS : ploidy	1, 180	357.551	<.0001
N : P : GS	0.112	0.291	0.385	0.7006	N : P : GS	1, 180	10.285	0.0016
N : P : ploidy	-0.083	0.291	-0.285	0.776	N : P : ploidy	1, 180	5.104	0.0251
N : GS : ploidy	-0.216	0.326	-0.663	0.5083	N : GS : ploidy	1, 180	12.012	0.0007
P : GS : ploidy	1.127	0.299	3.775	0.0002	P : GS : ploidy	1, 180	68.859	<.0001
N : P : GS : ploidy	1.095	0.411	2.662	0.0085	N : P : GS : ploidy	1, 180	7.088	0.0085

Table S2.12 continued

(f) R

	Estimate	Std. error	t value	Pr(>|t|)	ANOVA	DF	F-value	p-value
(Intercept)	1.630	0.115	14.152	<0.0001	(Intercept)	1, 180	3932.601	<.0001
N	0.178	0.167	1.069	0.2892	N	1, 60	0.668	0.4171
P	0.749	0.152	4.917	<0.0001	P	1, 60	13.456	0.0005
GS	-1.285	0.163	-7.886	<0.0001	GS	1, 180	197.863	<.0001
ploidy	0.276	0.163	1.695	0.0918	ploidy	1, 180	272.001	<.0001
N : P	-0.353	0.210	-1.682	0.0978	N : P	1, 60	0.122	0.7279
N : GS	-0.214	0.236	-0.909	0.3645	N : GS	1, 180	5.530	0.0198
P : GS	-0.828	0.216	-3.842	0.0002	P : GS	1, 180	2.649	0.1054
N : ploidy	0.002	0.236	0.009	0.9931	N : ploidy	1, 180	3.106	0.0797
P : ploidy	-0.834	0.216	-3.869	0.0002	P : ploidy	1, 180	0.173	0.6777
GS : ploidy	1.177	0.230	5.107	<0.0001	GS : ploidy	1, 180	520.549	<.0001
N : P : GS	0.246	0.297	0.828	0.4087	N : P : GS	1, 180	13.896	0.0003
N : P : ploidy	-0.150	0.297	-0.505	0.6144	N : P : ploidy	1, 180	3.397	0.067
N : GS : ploidy	-0.126	0.333	-0.379	0.7055	N : GS : ploidy	1, 180	14.417	0.0002
P : GS : ploidy	1.173	0.305	3.847	0.0002	P : GS : ploidy	1, 180	68.758	<.0001
N : P : GS : ploidy	1.074	0.420	2.557	0.0114	N : P : GS : ploidy	1, 180	6.537	0.0114

Appendix 2: Supporting Information Chapter 3

Supporting Figures

Supporting Tables

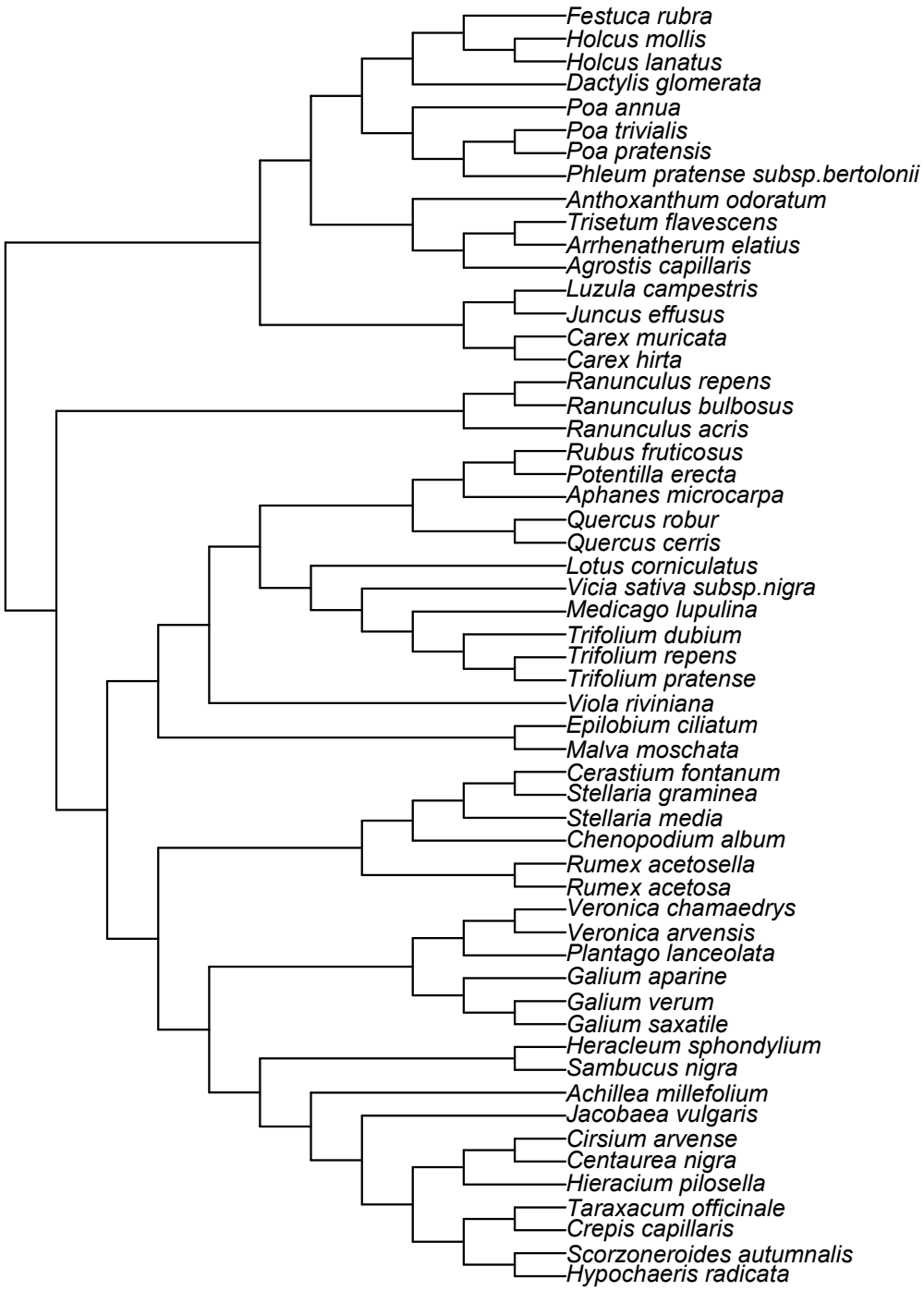


Figure S3. 1 Phylogenetic tree of the 56 species occurring amongst the 556 limed plots of Nash's Field, at Silwood Park.

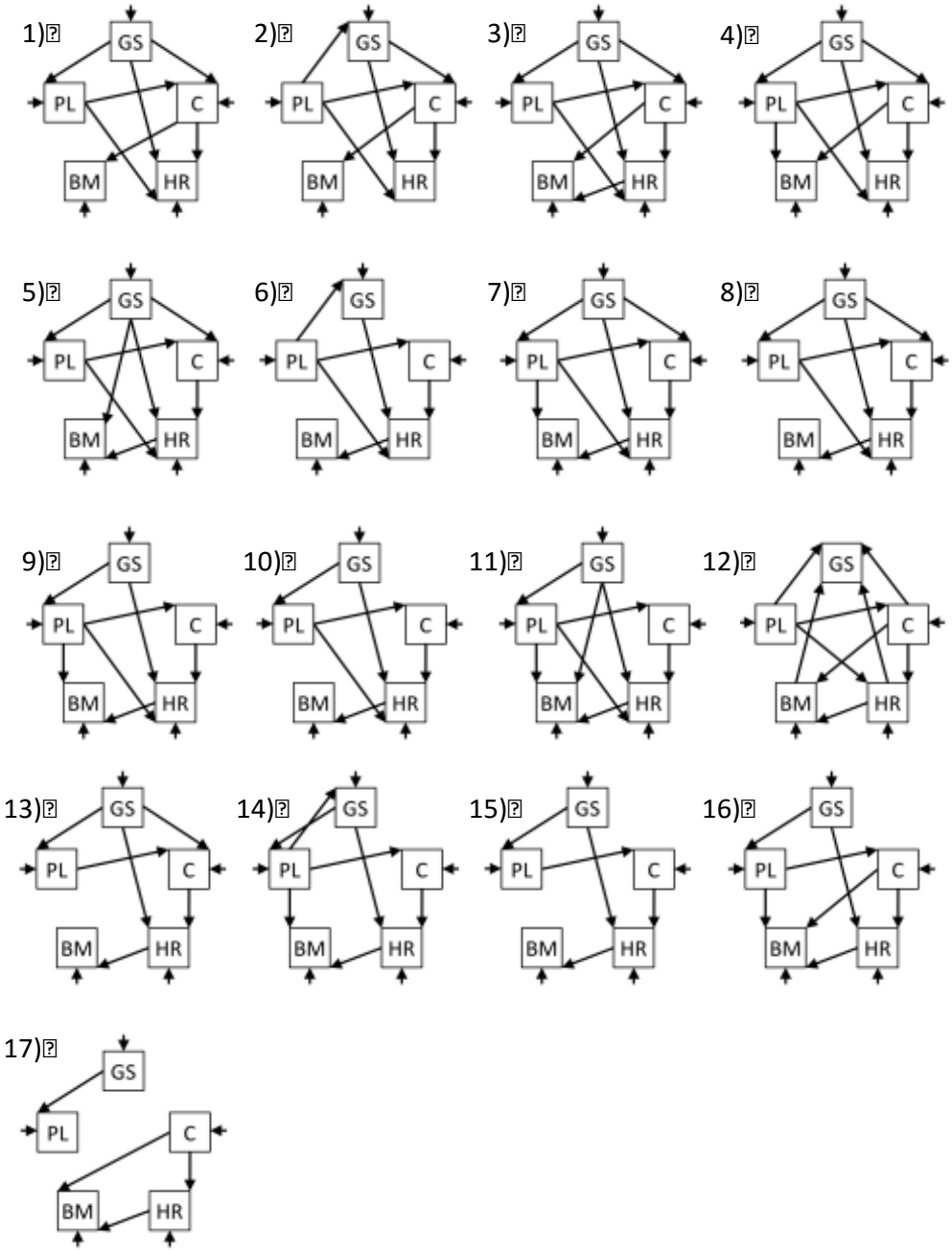


Figure S3. 2 Directed acyclic graphs representing path models investigating the effects exerted by the experiment on community parameters of genome size (GS), ploidy level (PL), competition (C), resistance to herbivory (HR), and total biomass (BM), and how these five traits are associated with each other. The small vertices (arrows) pointing towards each variable represent the effect of experimental treatment (herbivore exclusion, nitrogen, and phosphate input). One arrow originates from the experiment to each of the five community traits in all our model diagrams, with one exception (no. 12), in which we tested the conditional independence between the experiment and GS, see Table S3.6 for more details about path analysis and conditional independency tests).

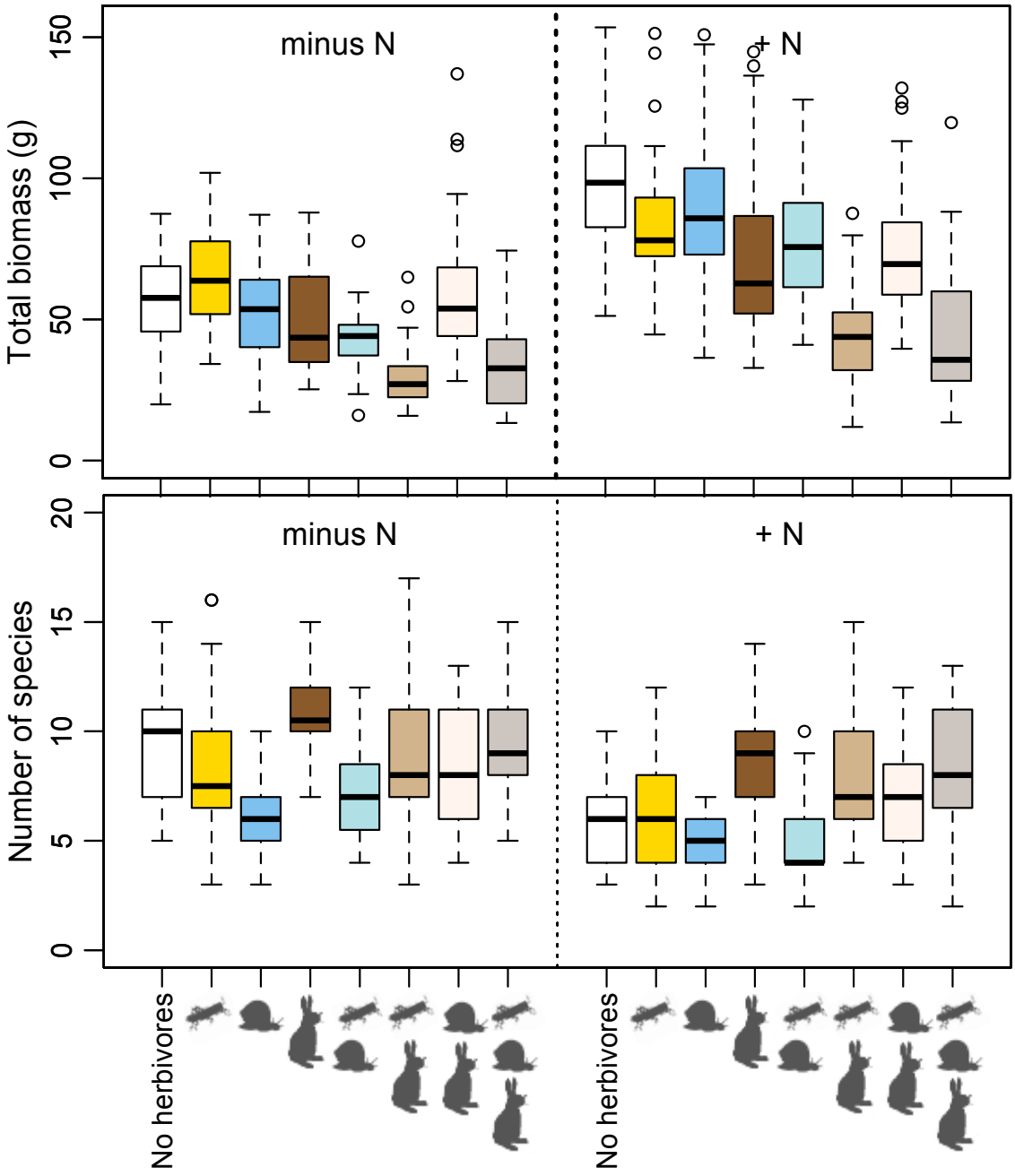


Figure S3.3 Total biomass (top); and total number of species (bottom) of each plot (n= 556), shown according to herbivore treatment (insects, molluscs, rabbits) and nitrogen (N) input. See also Table S3.11.

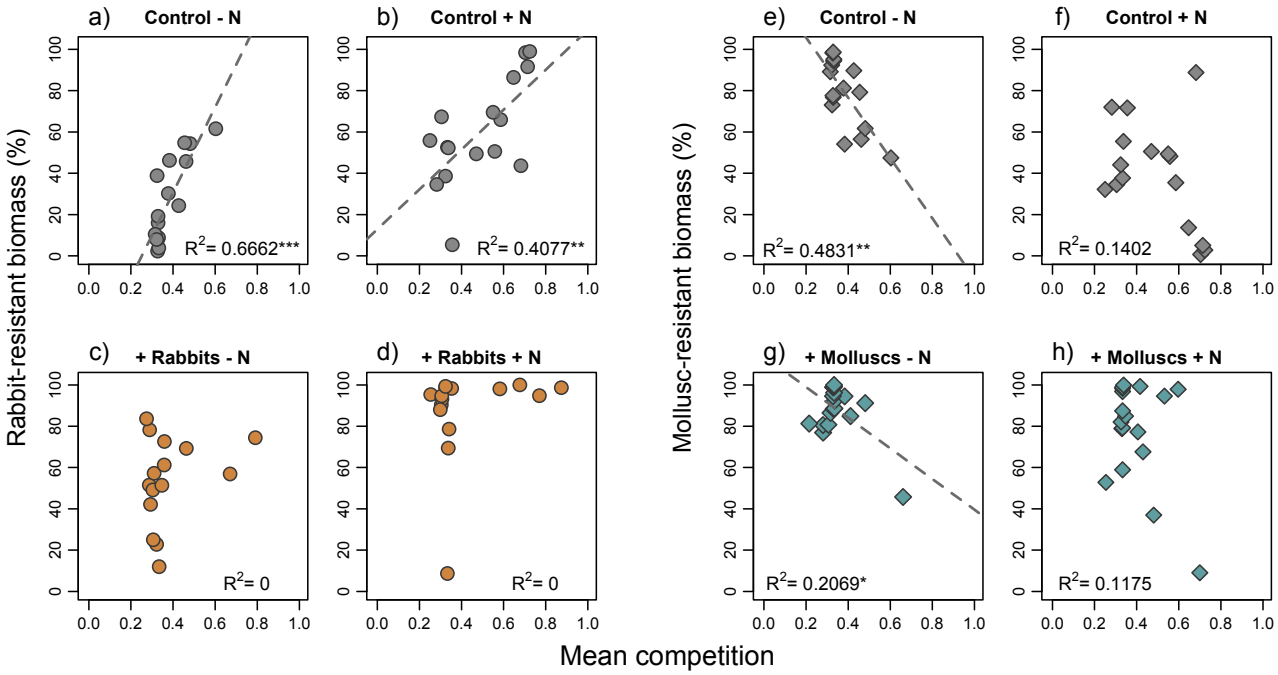


Figure S3.4 Associations between the biomass of rabbit (**a**-**d**) and mollusc (**e**-**f**) – resistant species and mean competition (Grime's C-strategy) under control plots (i.e. rabbit, mollusc, and insect- excluded plots) (**a**, **b**, **e**, **f**); + rabbit plots (**c**, **d**); and + mollusc plots (**g**, **h**). Within each herbivory type is +/- N treatment. The adjusted R^2 is shown in each plot. **a**) B =206.66, F(1, 14) = 30.94, p < 0.0001; **b**) B = 95.51, F(1, 14) = 11.32, p= 0.0046; **c**) B = 37.48, F(1, 13) = 1.00, p = 0.3353; **d**) B = 28.51, F(1, 13)= 0.8064, p = 0.3855; **e**) B= coef 146.00, F(1, 14) =15.30, p= 0.00163; **f**) B = -65.24, F(1, 14) = 3.45, p= 0.0846; **g**) B =-72.24, F(1, 16) = 7.00, p= 0.0176; **h**) B= -90.11, F(1, 16) =3.26, p= 0.0897.

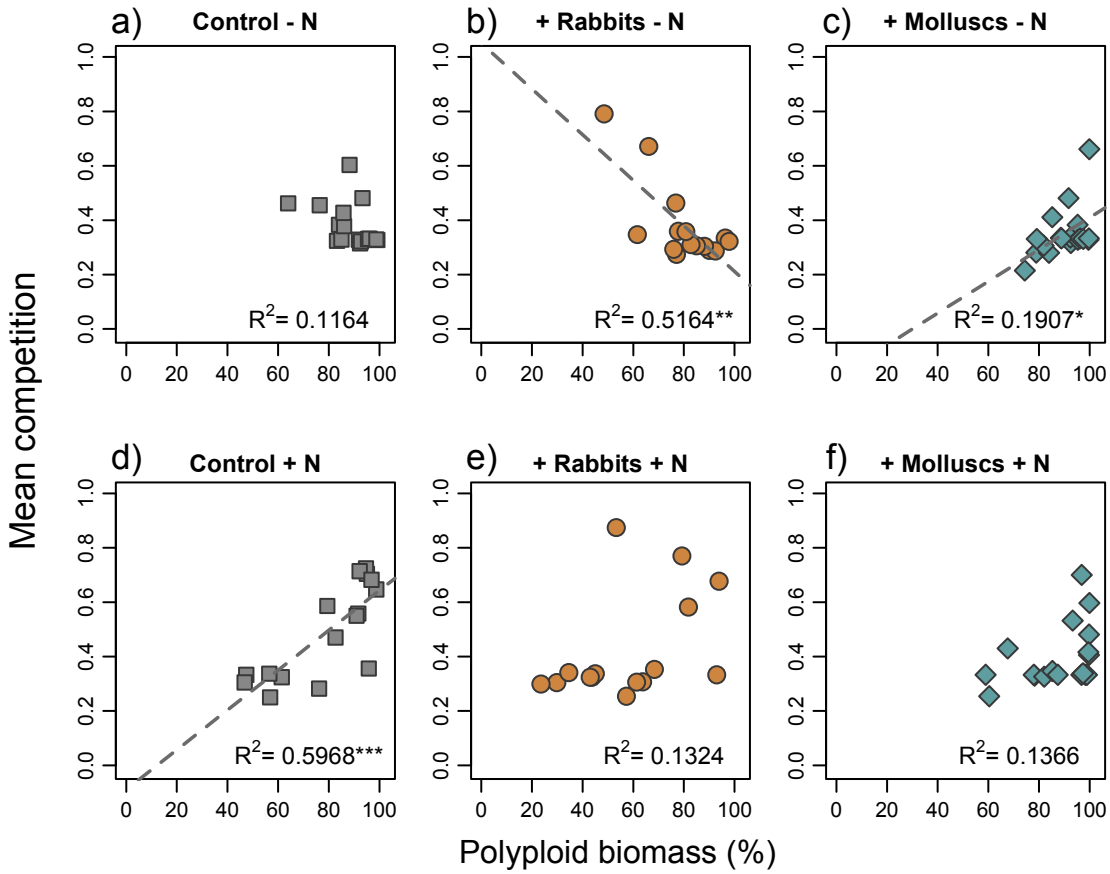


Figure S3.5 Scatter plots comparing the relationships between competition (Grime's C-strategy) and percent of total polyploidy biomass. The adjusted R^2 is shown in each plot. **a**) B = -0.004, F(1, 14) = 3.00, p = 0.1065; **b**) B = -0.008, F(1, 13) = 15.95, p= 0.00153; **c**) B = 0.006, F(1, 16) = 5.00, p = 0.03986; **d**) B = 0.007, F(1, 14)= 23.21, p = 0.00027; **e**) B= 0.004, F(1, 13) = 3.14, p= 0.1000; **f**) B = 0.0035, F(1, 16) = 3.69 , p= 0.07279.

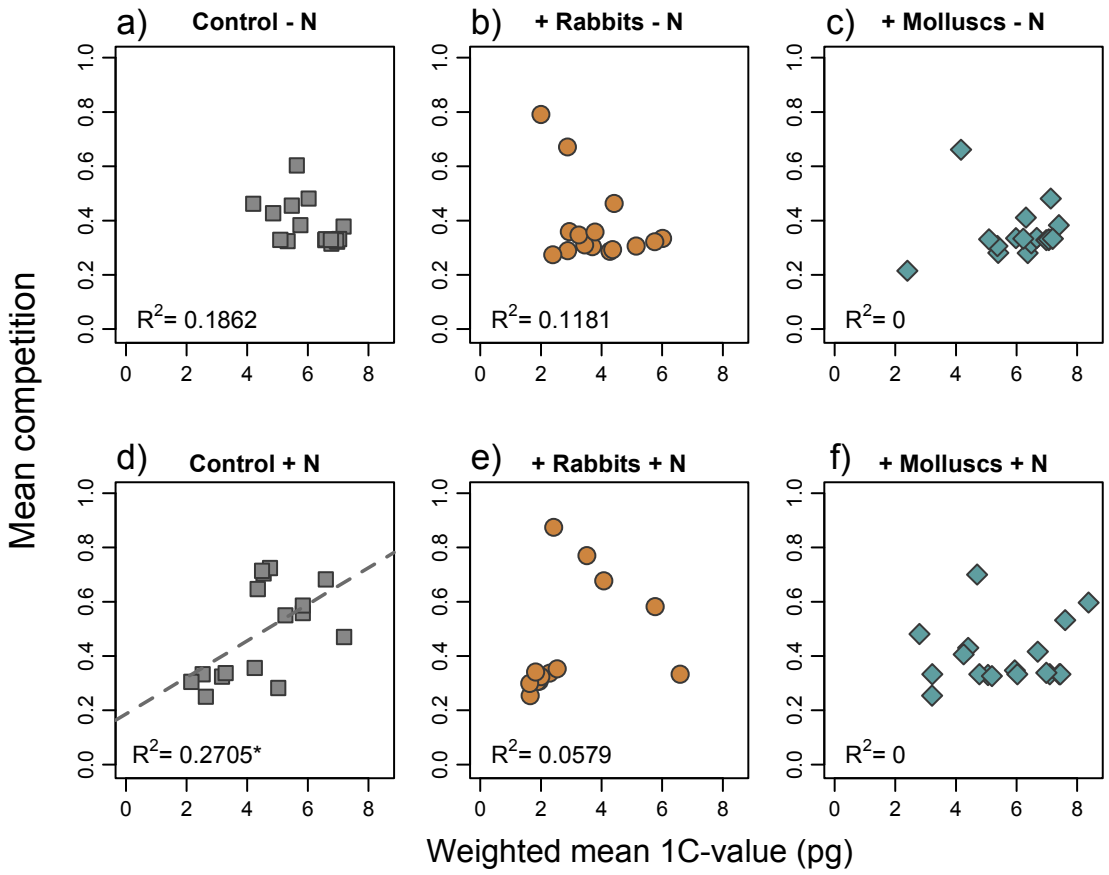


Figure S3.6 Scatter plots comparing the relationships between biomass-weighted mean GS and mean competition (Grime's C-strategy) with +/- N treatments on plots where all herbivores are excluded (control) (**a**, **d**); + rabbit plots (**b**, **e**); and + mollusc plots (**c**, **f**). The adjusted R^2 is shown in each plot. **a**) B = -0.044, F(1, 14) = 4.43, p = 0.05378; **b**) B = -0.054, F(1, 13) = 2.87, p= 0.1138; **c**) B = 0.002, F(1, 16) = 0.01, p = 0.9203; **d**) B = 0.067, F(1, 14)= 6.56, p = 0.0226.; **e**) B= 0.45, F(1, 13) = 1.86, p= 0.1958; **f**) B = 0.009, F(1, 16) = 0.30 , p= 0.5913.

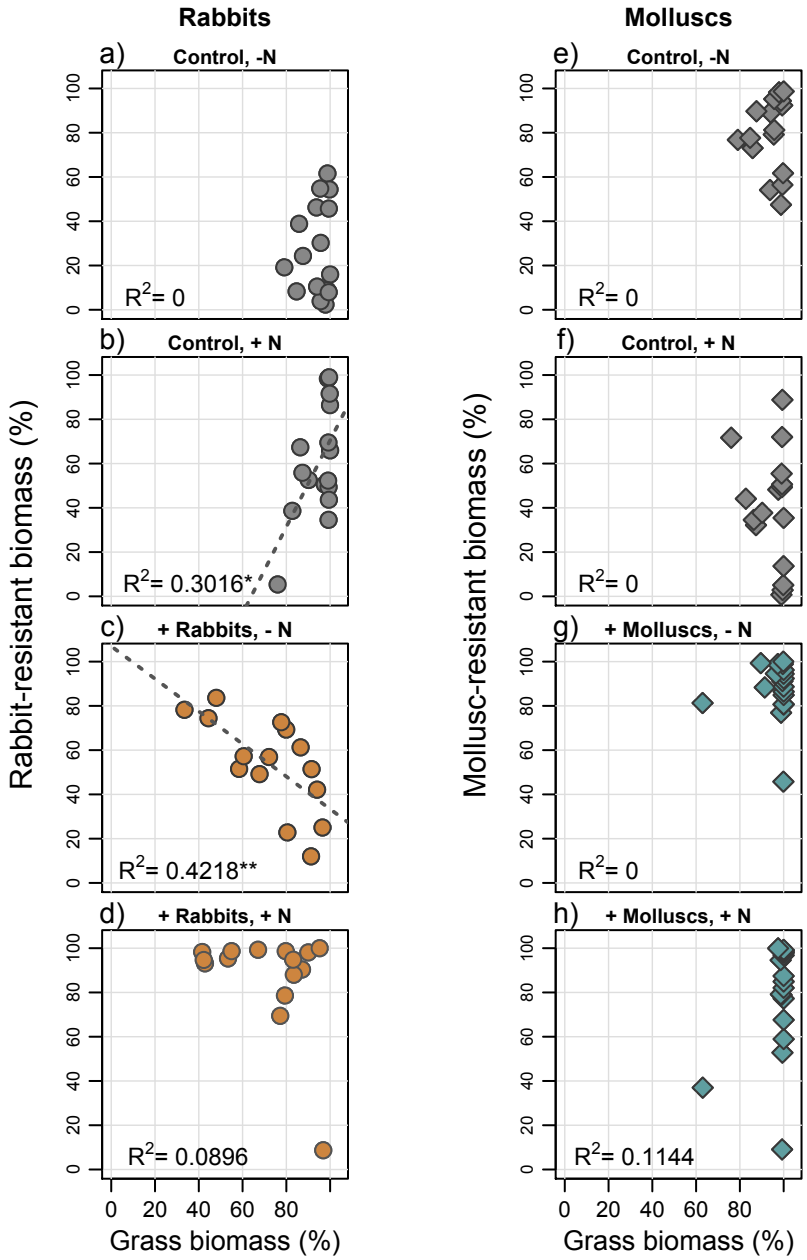


Figure S3.7 Herbivore-resistant biomass and grass biomass: in comparison to GS, grasses (functional group) is not a good predictor of tolerance to herbivory. Rabbits: **a**) control: B = 0.47, F(1, 14) = 0.31, p = 0.586; **b**) B = 1.95, f(1, 14) = 7.48, p = 0.0161; **c**) B = -0.74, F(1, 13) = 11.21; p = 0.0052; **d**) B = -0.46, F(1, 13) = 2.38, p = 0.1471. Molluscs: **e**) B = 0.046, F(1, 14) = 0, p = 0.9479; **f**) B = 0.75, F(1, 14) = 0.74, p = 0.4045; **g**) B = 0.05, F(1, 16) = 0.02, p = 0.8892; **h**) B = 1.17. F(1, 16) = 3.195. Adjusted R^2 is shown in each plot.

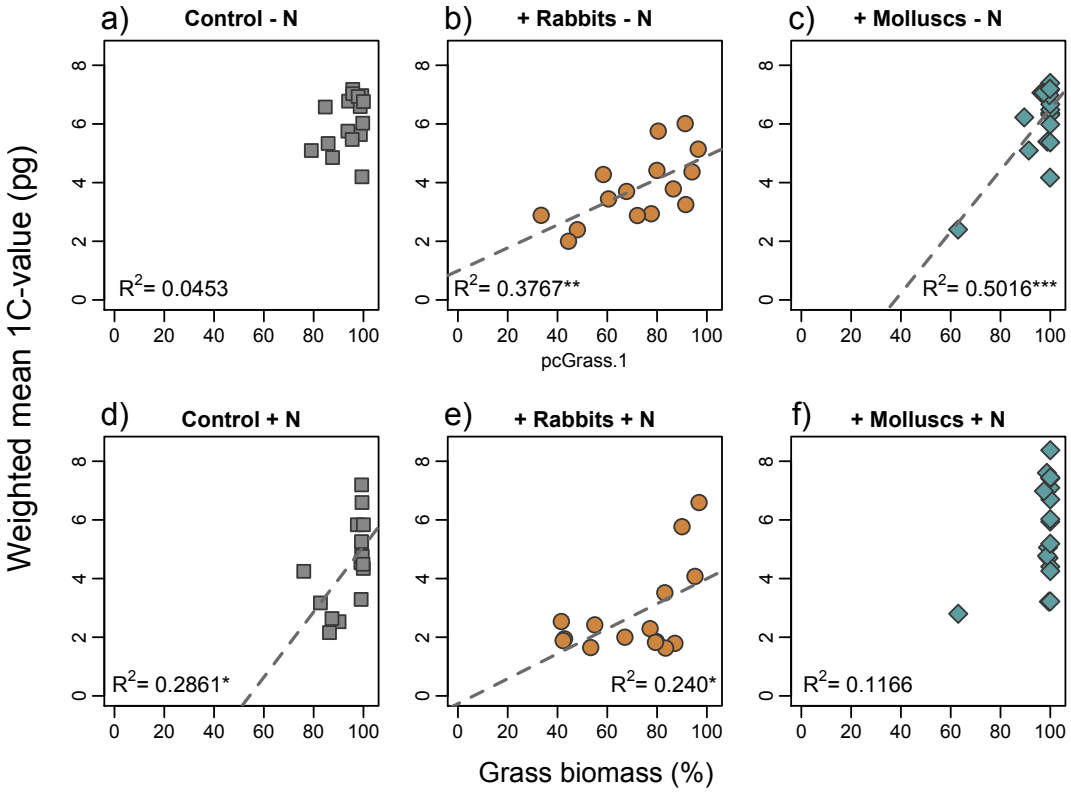


Figure S3.8 Abundance of grasses and weighted mean genome size are positively correlated on rabbit plots, particularly with N limitation. **a**) B = 0.046, F(1, 14) = 1.712, p = 1.712; **b**) B = 0.039, F(1, 13) = 9.46, p = 0.0088; **c**) B = 0.104, F(1, 16) = 18.11; p = 0.0006; **d**) B = 0.11, F(1, 14) = 7.01, p = 0.0191; **e**) B = 0.04, F(1, 13) = 5.416, p = 0.0367; **f**) B = 0.08, F(1, 16) = 3.24, p = 0.0906.

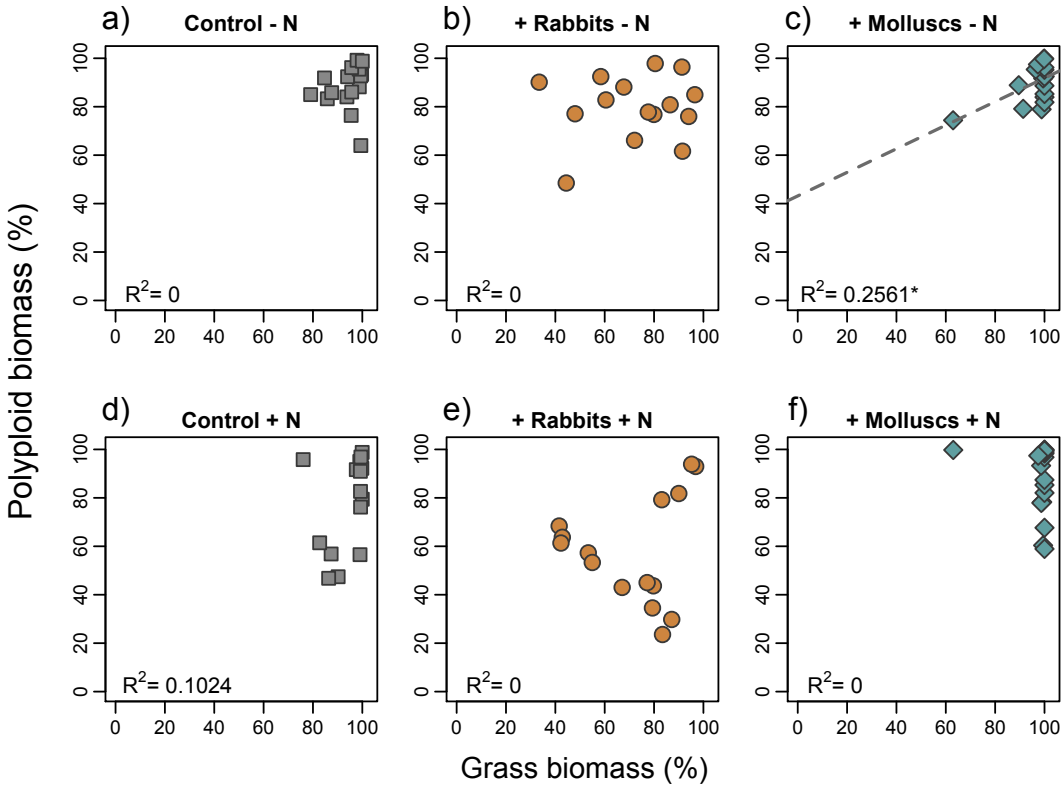


Figure S3.9 The total biomass attributed to the functional group of grasses shows very little correlation with the abundance of polyploids. **a**) B = 0.20, F(1, 14) = 0.30, p = 0.591; **b**) B = 0.68, f(1, 13) = 0.178, p = 0.6795; **c**) B = 0.49, F(1, 16) = 6.85; p = 0.0186; **d**) B = 0.996, F(1, 14) = 2.71, p = 0.1219; **e**) B = 0.14, F(1, 13) = 0.22, p = 0.6487; **f**) B = -0.35, F(1, 16) = 0.80, p = 0.3832.

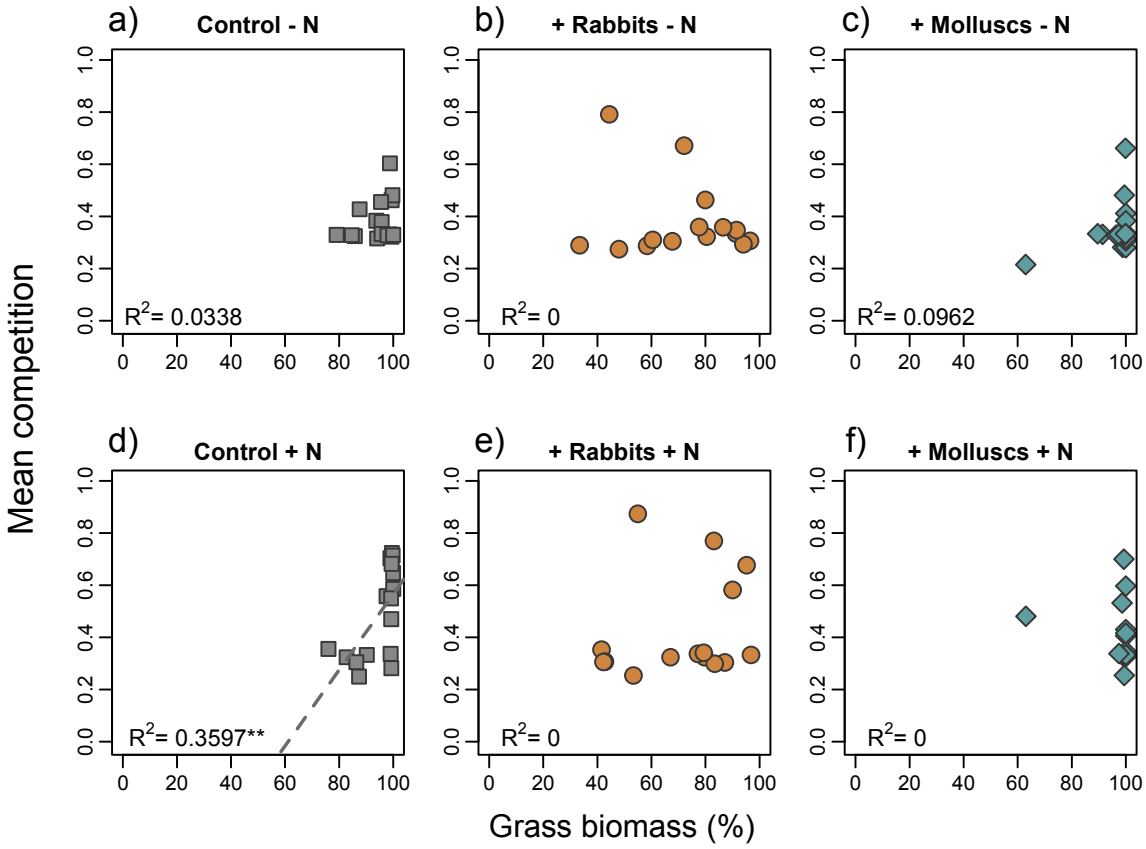


Figure S3.10 Under grazing pressure, functional group is not correlated with competition. **a**) B = 0.004, F(1, 14) = 0.24, p = 0.2373; **b**) B = -0.001, f(1, 13) = 0.56, p = 0.4691; **c**) B = 0.004, F(1, 16) = 2.81; p = 0.1131; **d**) B = 0.01, F(1, 14) = 9.427, p = 0.0083; **e**) B = 0.002, F(1, 13) = 0, p = 0.4985; **f**) B = -0.002, F(1, 16) = 0.574, p = 0.4597.

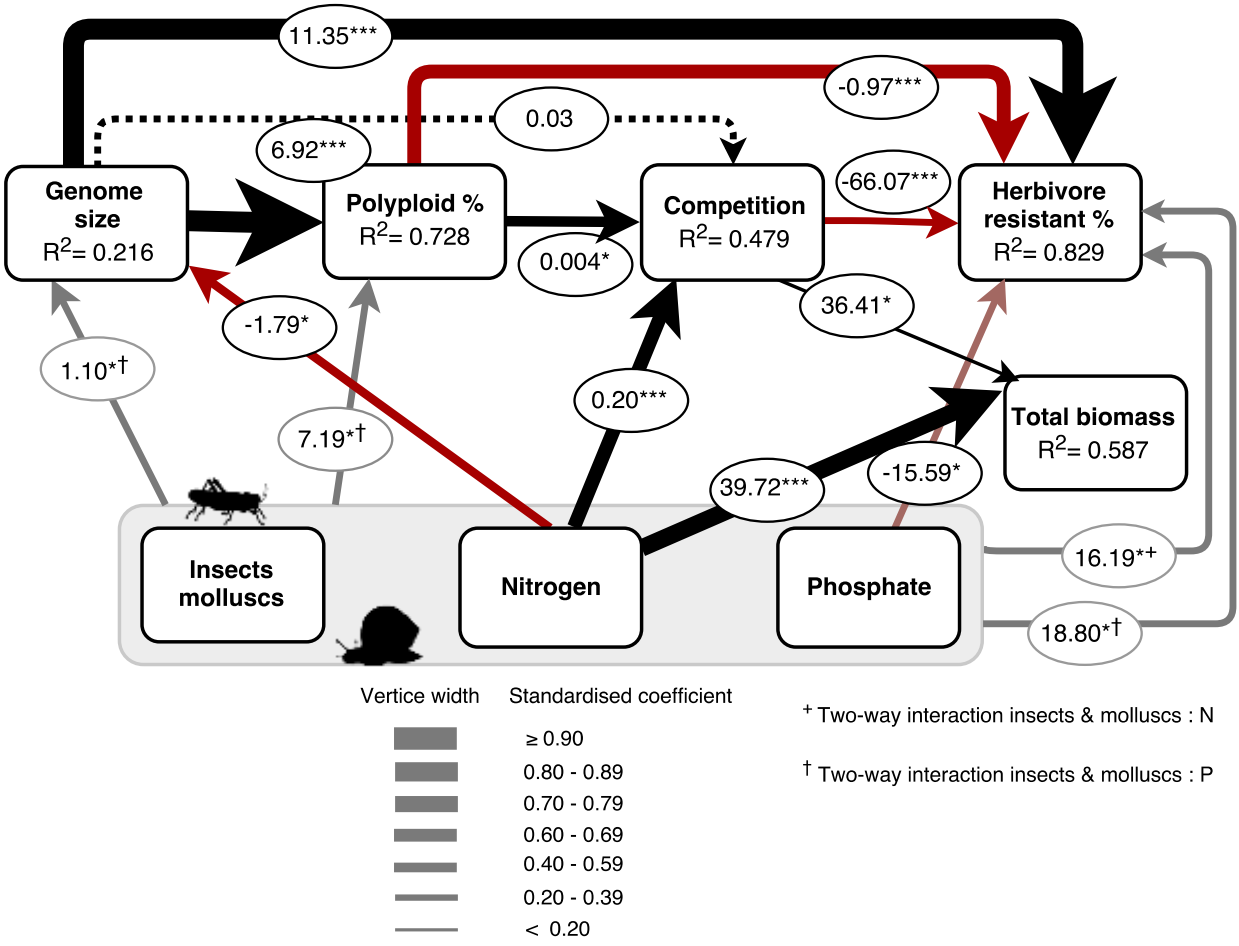


Figure S3.11 Path analysis showing effects of insect and mollusc grazing (i.e. plots with both insects and molluscs). Unstandardised partial regression coefficients are shown for each path. A dotted line shows a non-significant regression coefficient. Vertice (arrow) widths correspond to standardized coefficients, see also legend below. Non-significant effects of the experiment are not shown. P-values are represented as follows: *** < 0.0001, ** < 0.001, * < 0.05.

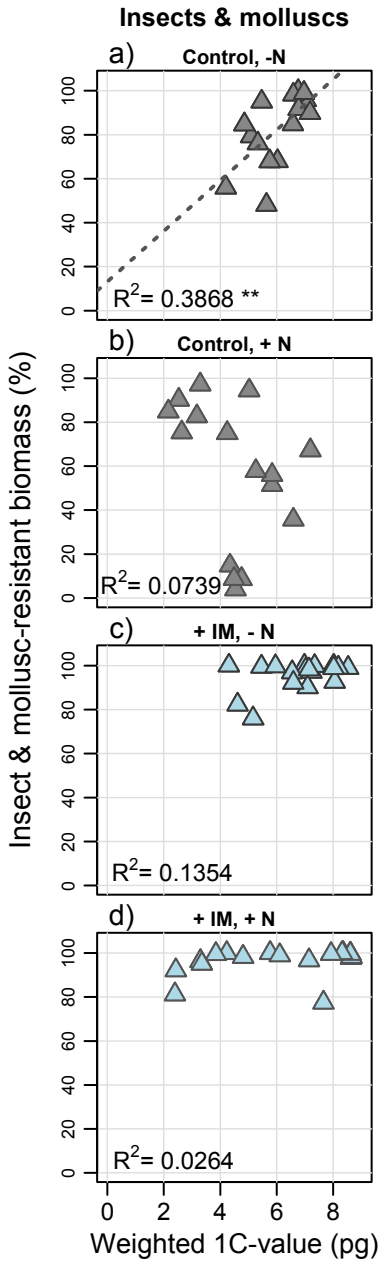


Figure S3.12 Relationship between weighted mean GS and percent biomass of: insect and mollusc-resistant species under four different experimental treatments: 1) control (i.e. rabbit, mollusc, and insect excluded plots) minus nitrogen (N); 2) control + N; 3) insect and molluscs - N; 4) insects and molluscs + N. The adjusted R is shown in each plot. **a**) B - 11.508, F(1, 14) = 10.46, p= 0.0060; **b**) B - -8.214, F(1, 14) = 2.197, p= 0.1605; **c**) B = 2.331, F(1, 16) = 3.663, p = 0.0737; **d**) B = 0.7933, F(1, 16)= 1.461, p = 0.2444. p-values are shown as follows : *** < 0.0001, ** < 0.001, * < 0.05.

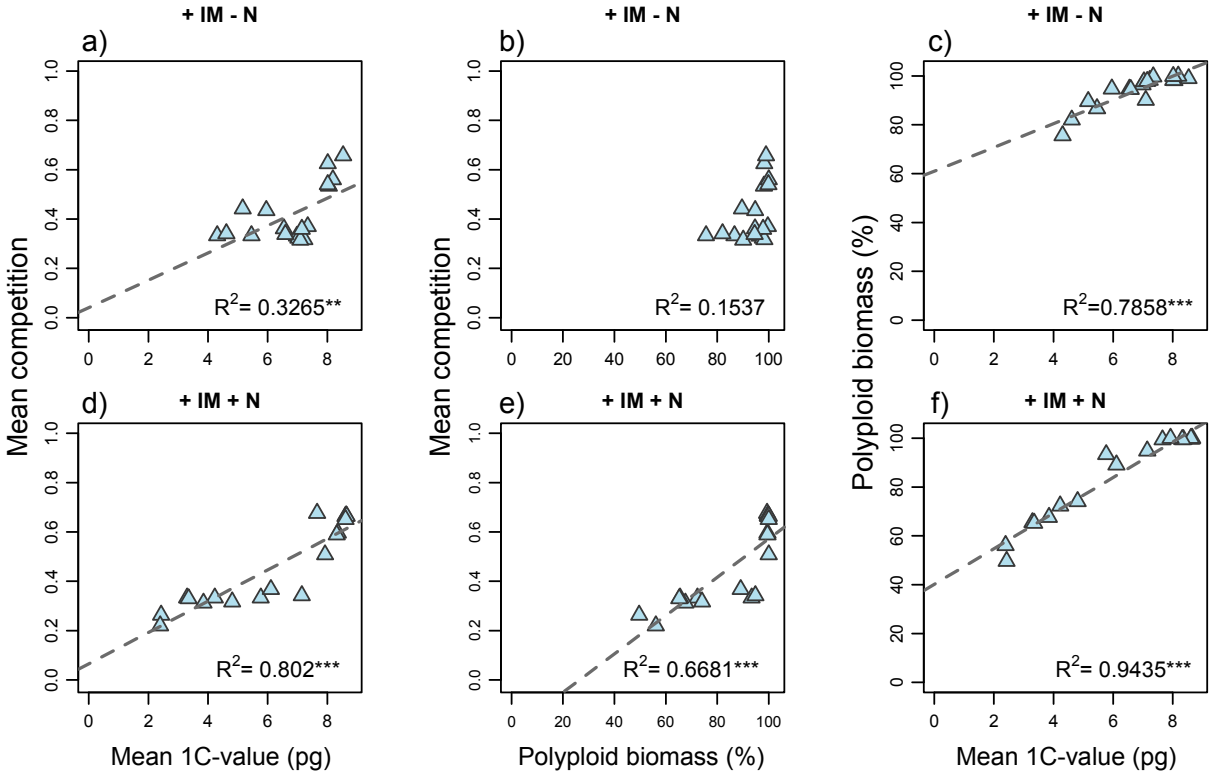


Figure S3.13 Scatter plots showing the associations between competition and mean biomass-weighted GS on control plots and on plots with both insect and mollusc (**IM**) herbivory (i.e. fenced plots without insecticides and molluscicides), with +/- N treatment. Linear regression statistics for a) and c) are reported above (Fig. S3.7).

a) B = 0.055, F(1, 16) = 9.24, p = 0.0078; **b**) B = 0.007, F(1, 16)= 4.09, p= 0.06026; **c**) coef= 4.87, F(1, 16)= 63.37, p < 0.0001; **d**) B = 0.06, F(1, 16) = 69.84, p < 0.0001; **e**) B = 0.007, F(1, 16)= 35.23, p< 0.0001; **f**) B= 7.29, F(1, 16) = 284.7, p < 0.0001. The adjusted R is shown.

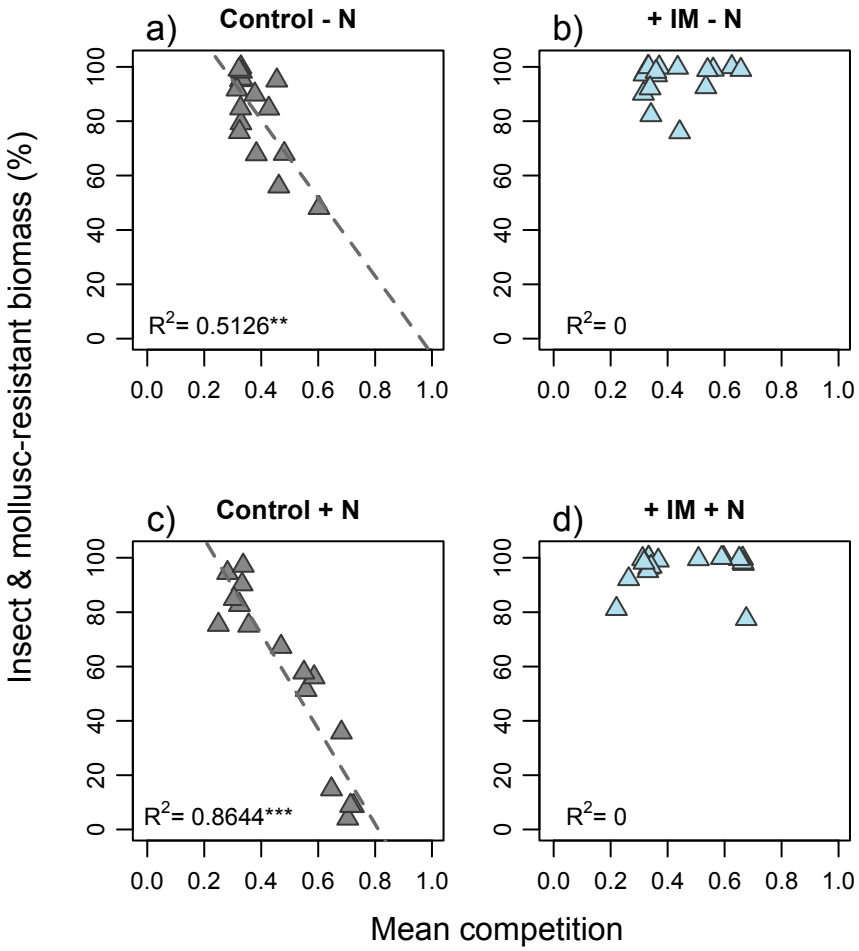


Figure S3.14 The effect of competition on biomass of species resistant to insect & mollusk (IM) herbivory. **a**) control - N : B = -143.95, F(1, 14)= 16.77, p= 0.00109; **b**) + IM - N : B= 7.90, F(1, 16) = 0.29, p = 0.5960; **c**) control + N: B = -174.862, F(1, 14) = 96.6, p < 0.00001; **d**) + IM + N : B = 3.536, F(1, 16)= 0.13, p= 0.7205. The adjusted R^2 is shown.

Table S3. 1 Flow cytometry on 45 species collected at Silwood Park, showing the estimated 1C- value (pg) obtained, number of plants measured (n), standard deviation in 1C-value, target and standard coefficients of variation (CoV).

Taxa	1C-value (pg)	n	sdev	target CoV	standard CoV
Achillea_millefolium	7.98	3	0.03	3.16	3.40
Agrostis_capillaris	3.60	8	0.25	4.43	4.37
Agrostis_stolonifera	3.12	2	0.74	6.86	5.27
Anthoxanthum_odoratum	7.28	3	0.36	4.05	4.27
Arrhenatherum_elatius	8.61	4	0.47	3.15	4.56
Bromus_sterilis	3.39	3	0.20	5.12	5.76
Carex_muricata	0.38	7	0.01	6.62	4.81
Centaurea_nigra	2.13	2	0.29	7.17	6.23
Cerastium_fontanum	3.23	2	0.20	4.29	3.68
Chenopodium_album	1.95	1	NA	6.76	6.84
Cirsium_arvense	1.48	1	NA	4.64	3.74
Cirsium_vulgare	2.96	1	NA	6.45	8.12
Crepis_capillaris	2.45	2	0.13	8.50	5.81
Dactylis_glomerata	4.44	2	0.21	4.60	5.20
Festuca_rubra	7.31	2	0.02	5.25	5.46
Galium_aparine	1.11	1	NA	6.88	5.14
Galium_saxatile	1.72	1	NA	4.21	2.44
Galium_verum	2.25	1	NA	6.99	8.67
Heracleum_sphondylium	2.46	2	0.15	5.09	5.26
Hieracium_pilosella	3.52	2	0.05	6.46	6.95
Holcus_lanatus	1.70	1	NA	4.14	2.94
Holcus_mollis	4.03	3	0.04	6.30	7.31
Jacobaea_vulgaris	2.30	3	0.11	5.54	4.46
Juncus_effusus	0.28	2	0.02	5.37	4.61
Lolium_perenne	2.72	2	0.07	4.92	4.16
Lotus_corniculatus	1.30	4	0.05	4.75	4.41
Luzula_campestris	0.39	2	0.01	5.54	4.32
Medicago_lupulina	0.55	1	NA	15.07	4.82
Papaver_dubium	3.72	3	0.14	3.80	3.99
Phleum_pratense_bertolonii	1.88	3	0.08	3.13	3.54
Plantago_lanceolata	1.43	2	0.03	4.99	4.79
Poa_trivialis	2.01	4	0.02	4.41	3.68
Prunella_vulgaris	0.76	2	0.05	15.65	5.57
Ranunculus_acris	4.98	2	0.13	3.79	4.24

Table S3.1 continued Taxa	1C-value (p	n	sdev	target CoV	standard CoV
Ranunculus_repens	11.06	2	0.14	5.68	5.69
Rubus_sp	0.87	1	NA	19.00	6.72
Rumex_acetosella	1.07	2	0.01	7.50	6.00
Sonchus_asper	0.86	2	0.03	8.12	3.55
Stellaria_graminea	1.01	7	0.07	4.15	4.21
Trifolium_repens	1.12	2	0.02	6.41	6.11
Urtica_dioica	1.32	2	0.09	6.54	4.90
Verbascum_nigrum	0.42	1	NA	7.08	5.28
Veronica_chamaedrys	2.16	2	0.02	5.02	3.80
Vicia_sativa_nigra	2.21	2	0.02	6.71	5.80
Viola_riviniana	1.47	2	0.15	6.93	4.08

Table S3. 2 Species list showing the 1C-values (pg) attributed to each species (second column) in our analyses. The asterisks represent 1C-values that were estimated from samples collected on site, with flow ctyomtery. Columns 3 to 5 contain information on 1C-value, and the associated ploidy level and number of chromosomes for each taxon, which are held in the Plant DNA C-values database (Bennett and Leitch 2012). The last column shows published chromosome counts, obtained from The Chromosome Counts Database (CCDB) (Rice et al. 2105).

Taxon	1C-value (pg)	1C-value (Kew database)	Ploidy level	Chromosome count	Known chromosome counts
Acer pseudoplatanus	1.35	1.35	4	52	52
Achillea millefolium	7.98*	7.65	6	54	18, 27, 36, 54, 45, 54, 63, 72, 74
Agrostis capillaris	3.6*	3.53, 4.65	4, 6	2	28, 42
Agrostis stolonifera	3.50	3.50	na	na	28, 42
Alopecurus pratensis	6.80	6.80	na	na	28
Angelica archangelica	3.23	3.23	2	na	22
Anthoxanthum odoratum	7.28*	5.90	4	20	10, 20
Aphanes microcarpa	0.58	na (0.58)	na (6)	48	16, 48
Arrhenatherum elatius	8.61*	7.98	4	28	28
Betula pendula	0.50	(na) 0.50	2	na	28 (56)
Bromus hordeaceus	9.18	9.18	4	28	28
Bromus sterilis	3.35	3.35	2	14	14, 28
Carex hirta	0.53	(na) 0.30	na	na	112
Carex muricata	0.38*	(na) 0.30	na	na	50, 52-56, 58
Centaurea nigra	1.80	1.80	na	na	44
Cerastium fontanum	3.23*	2.93	16	144	126, 144
Chenopodium album	1.63	0.77, 1.63, 2.33	2, 4, 6	18	18, 36, 54

Table S2.2 continued

Taxon	1C-value (pg)	1C-value (Kew database)	Ploidy level	Chromosome count	Known chromosome counts
Cirsium arvense	1.42	1.42	2	34	34 (-36), 68
Cirsium vulgare	2.77	2.77	4	68	(34), 68
Conopodium majus	0.83	(na) 0.83	na	na	22
Conyza canadensis	0.45	0.45	2	na	18, (54)
Crataegus monogyna	0.76	0.76	2	34	34, (51)
Crepis capillaris	2.10	2.10	2	6	6
Dactylis glomerata	4.40	3.30, 4.40, 4.40	2, 4, 6	14, 28, 42	14, 28, (42)
Danthonia decumbens	2.95	2.95	4	36	(24), 36
Deschampsia cespitosa	5.22	5.22, 9.00	2, 4	26, 52	26, 52
Epilobium ciliatum	0.53	0.53	2	36	36
Festuca rubra	7.31*	4.73, 8.49	6, 8	42, 56	(28), 42, (46)
Fraxinus excelsior	0.98	0.98	2	46	46
Galium aparine	1.03	1.03	na	na	(42, 48, 62, 64), 66, (68)
Galium saxatile	1.45	1.45	4	44	(22), 44
Galium verum	1.89	1.89	4	44	(22), 44
Heracleum sphondylium	2.19	2.19	2	22	22
Hieracium pilosella	3.45	3.45	4	36	(18), 36, (45, 54, 63)
Holcus lanatus	1.7*	1.70	2	14	14
Holcus mollis	4.03*	2.78, 4.10	4, 5	28, 35	(14), 28, (35, 42, 49)
Hypericum humifusum	0.50	0.15	na	na	16
Hypochaeris radicata	1.34	1.34	8	2	8

Table S2.2 continued

Taxon	1C-value (pg)	1C-value (Kew database)	Ploidy level	Chromosome count	Known chromosome counts
Jacobaea vulgaris	2.25	2.25	4	40	40, (80)
Juncus effusus	0.30	0.30	2	46	40, 42
Lactuca serriola	1.85	1.85	2	18	18
Lolium perenne	2.72*	2.76	2	14	14
Lotus corniculatus	1.3*	0.48, 1.05	4	24	(12), 24, (42)
Luzula campestris	0.49	0.49	2	12	12
Malva moschata	1.10	1.00	6	42	42
Medicago lupulina	0.65	0.65	2	16	16, (32)
Papaver dubium	3.72*	3.73, 4.50	2, 4	14, 28	14, 28, 42
Phleum pratense subsp.bertolonii	1.88*	1.70	2	14	(14, 28), 42
Plantago lanceolata	1.43*	1.20	2	12	12, (24)
Poa annua	2.88	2.88	4	28	(14), 28
Poa pratensis	4.24	4.24	na	na	28, 36, 42-98
Poa trivialis	2.01*	2.83	2	14	14, (28)
Potentilla erecta	0.45	0.45	na	28	28
Prunella vulgaris	0.65	0.65	2	28	28
Prunus avium	0.35	0.35	na	na	16
Quercus cerris	0.95	0.95	2	24	24
Quercus robur	0.93	0.93	2	24	24
Ranunculus acris	4.98*	4.45	2	14	14, (28)
Ranunculus bulbosus	5.63	5.63	2	16	16
Ranunculus repens	11.20	11.2	4	32	32

Table S2.2 continued

Taxon	1C-value (pg)	1C-value (Kew database)	Ploidy level	Chromosome count	Known chromosome counts
Rubus sp	0.70	0.7	na	na	14, (28)
Rumex acetosa	1.65	1.65	2	14	14, (15)
Rumex acetosella	1.68	1.68	4	42	14, 28, 42
Sambucus nigra	15.25	15.25	2	36	36
Sanguisorba minor	0.55	0.55	4	28	28
Scorzoneroides autumnalis	1.16	1.16	2	16	12
Sonchus asper	1.85	1.85	2	18	18
Stellaria graminea	1.01*	na	na	na	26, 39, 52
Stellaria media	1.05	1.05	7	42	(24), 40, 42,44
Taraxacum officinale	1.28	1.28	2	16	24, (26, 27, 32, 40, 44, 48)
Trifolium dubium	0.73	0.73	4	28	28, 32
Trifolium pratense	0.43	0.43	2	14	14
Trifolium repens	1.12	1.12	4	32	(16), 32
Trisetum flavescens	2.55	2.55	na	na	12, (24, 28
Urtica dioica	1.58	1.58	4	52	(26), 48, 52
Verbascum nigrum	0.42*	0.40	na	na	30
Veronica arvensis	0.33	0.33	2	18	16, (18)
Veronica chamaedrys	2.16*	1.49	4	32	32
Veronica serpyllifolia	0.44	0.44	2	14	14
Vicia sativa subsp.nigra	2.25	2.25	2	12	12, (14)
Viola riviniana	1.35	1.35	4	40	40

Table S3. 3 Accessions obtained from Genbank (Benson et al. 2015), used in the estimation of phylogenetic trees.

Taxon	matK	rbcL
Acer_pseudoplatanus	KJ204427.1	FN689357.1
Achillea_millefolium	HM850607.1	JX848399.1
Agrostis_capillaris	JN895337.1	JN891522.1
Agrostis_stolonifera	KJ529328.1	KJ204295.1
Alopecurus_pratensis	HM850564.1	HM849759.1
Angelica_archangelica	GQ248079.1	GQ248549.1
Anthoxanthum_odoratum	HM850562.1	HM849780.1
Aphanes_microcarpa	HM850684.1	HM849782.1
Arrhenatherum_elatius	KJ529335.1	AY395529.1
Betula_pendula	AM889694.1	KF418943.1
Bromus_hordeaceus	KJ529322.1	HM849826.1
Bromus_sterilis	JN895117.1	JN892201.1
Carex_hirta	JN895308.1	KJ841178.1
Carex_muricata	JN894492.1	JN891205.1
Centaurea_nigra	JN895499.1	JN893384.1
Cerastium_fontanum	HM850786.1	HM849881.1
Chenopodium_album	KJ840894.1	KJ204316.1
Cirsium_arvense	KJ204457.1	KJ841240.1
Cirsium_vulgare	HM850623.1	HM849899.1
Conopodium_majus	JN895810.1	JN893624.1
Conyza_canadensis	HQ593246.1	KJ204321.1
Crataegus_monogyna	KJ204463.1	KJ204324.1
Crepis_capillaris	JN895402.1	HM849923.1
Dactylis_glomerata	HM850569.1	AY395535.1
Danthonia_decumbens	KJ529309.1	JN892157.1
Deschampsia_cespitosa	JN894900.1	JX848495.1
Epilobium_ciliatum	KM212071.1	KF997326.1
Festuca_rubra	HQ593297.1	JN891471.1
Fraxinus_excelsior	JN895188.1	KJ204344.1
Galium_aparine	HM850825.1	KJ841334.1
Galium_saxatile	HM850829.1	HM850022.1
Galium_verum	JN893877.1	JN892891.1
Heracleum_sphondylium	JN894476.1	JN893491.1

Table S3.3 continued

Taxon	matK	rbcL
Hieracium_pilosella	HE970711.1	JN891685.1
Holcus_lanatus	JN894527.1	JN892327.1
Holcus_mollis	JN895365.1	JN892525.1
Hypericum_humifusum	HM850931.1	HM850063.1
Hypochaeris_radicata	HM850666.1	HM850069.1
Jacobaea_vulgaris	JN895164.1	HE963662.1
Juncus_effusus	HQ593334.1	HQ590146.1
Lactuca_serriola	HQ593336.1	KJ204362.1
Lolium_perenne	HM850533.1	JN893059.1
Lotus_corniculatus	HM049505.1	JN892127.1
Luzula_campestris	JN895446.1	HM850146.1
Malva_moschata	KJ204506.1	KJ204372.1
Medicago_lupilina	KJ204508.1	KJ204374.1
Papaver_dubium	HM851026.1	HM850229.1
Phleum_pratense_subsp.bertolonii	JN894629.1	JN893804.1
Plantago_lanceolata	HE966968.1	JN893615.1
Poa_annua	KJ529344.1	KJ599225.1
Poa_pratensis	JN966444.1	JN965752.1
Poa_trivialis	HM850517.1	JN893080.1
Potentilla_erecta	HM850688.1	HM850285.1
Prunella_vulgaris	HM850805.1	AY395556.1
Prunus_avium	KJ204527.1	HQ235394.1
Quercus_cerris	FN675335.1	AB125017.1
Quercus_robur	JN895518.1	FN675735.1
Ranunculus_acris	JN894744.1	JN965795.1
Ranunculus_bulbosus	HM851057.1	JN892326.1
Ranunculus_repens	HM851062.1	HM850298.1
Rubus_fruticosus	JN894438.1	JN891407.1
Rumex_acetosa	JN895619.1	JN893396.1
Rumex_acetosella	KJ746190.1	D86290.1
Sambucus_nigra	JQ412285.1	KC584884.1
Sanguisorba_minor	HM850691.1	JN892329.1
Scorzoneroides_autumnalis	JN894632.1	JN890754.1

Table S3.3 continued

Taxon	matK	rbcL
Sonchus_asper	HM850661.1	KJ841589.1
Stellaria_graminea	JN895064.1	KJ841601.1
Stellaria_media	AY936299.1	M62570.1
Taraxacum_officinale	FJ395377.1	JX520956.1
Trifolium_dubium	KJ204549.1	KJ204416.1
Trifolium_pratense	JN895372.1	JN893083.1
Trifolium_repens	HE967014.1	JN892960.1
Trisetum_flavescens	KJ204553.1	JN893258.1
Urtica_dioica	HQ593482.1	AF500361.1
Verbascum_nigrum	JN896246.1	JN893706.1
Veronica_arvensis	KJ841039.1	KJ841651.1
Veronica_chamaedrys	JN894843.1	JN891876.1
Veronica_serpyllifolia	HM851040.1	KJ841654.1
Vicia_sativa_subsp.nigra	HM851165.1	HM850456.1
Viola_riviniana	KJ747843.1	KJ595802.1

Table S3.4 Species list showing family, order, C-S-R type (Grime 1977), whether a species was scored as resistant (=1) to rabbit, mollusc, and insect + mollusc (I+M) grazing, and as diploid (= 0) or polyploid (= 1).

Taxon	Family (n=25)	C-S-R type	Rabbit resistant (n=22)	Mollusc resistant (n=19)	Insect & mollusc resistant (n=18)	Polyploid (n= 37)
Acer pseudoplatanus	Sapindaceae	C/SC	0	0	0	1
Achillea millefolium	Asteraceae	CSR	0	0	0	1
Agrostis capillaris	Poaceae	CSR	1	1	1	1
Agrostis stolonifera	Poaceae	CR	0	0	0	1
Alopecurus pratensis	Poaceae	C/CSR	0	0	0	1
Angelica archangelica	Apiaceae	CS	0	0	0	0
Anthoxanthum odoratum	Poaceae	SR/CSR	1	1	0	1
Aphanes microcarpa	Rosaceae	SR	0	0	0	1
Arrhenatherum elatius	Poaceae	C/CSR	1	1	1	1
Betula pendula	Betulaceae	C/SC	0	0	0	0
Bromus hordeaceus	Poaceae	R/CR	0	0	0	1
Bromus sterilis	Poaceae	R/CR	0	0	0	1
Carex hirta	Cyperaceae	C/CSR	0	0	1	0
Carex muricata	Cyperaceae	S/CSR	0	0	0	0
Centaurea nigra	Asteraceae	CSR	0	1	1	0
Cerastium fontanum	Caryophyllaceae	R/CSR	1	0	1	1
Chenopodium album	Amaranthaceae	CR	1	0	0	0
Cirsium arvense	Asteraceae	C	1	1	0	0
Cirsium vulgare	Asteraceae	CR	0	0	0	1
Conopodium majus	Apiaceae	SR	0	0	0	0
Conyza canadensis	Asteraceae	R/CR	0	0	0	0
Crataegus monogyna	Rosaceae	SC	0	0	0	0
Crepis capillaris	Asteraceae	R/SR	1	1	0	0
Dactylis glomerata	Poaceae	C/CSR	0	1	0	1
Danthonia decumbens	Poaceae	S/CSR	0	0	0	1
Deschampsia cespitosa	Poaceae	SC/CSR	0	0	0	0
Epilobium ciliatum	Onagraceae	R/CSR	0	0	0	0
Festuca rubra	Poaceae	CSR	0	1	1	1
Fraxinus excelsior	Oleaceae	C/SC	0	0	0	0
Galium aparine	Rubiaceae	CR	1	0	0	1

Table S3.4 continued

Taxon	Family	C-S-R type	Rabbit resistant	Mollusc resistant	I+M resistant	Polyploid
Galium saxatile	Rubiaceae	S/CSR	0	1	1	1
Galium verum	Rubiaceae	SC/CSR	0	0	0	1
Heracleum sphondylium	Apiaceae	C/CSR	0	0	0	0
Hieracium pilosella	Asteraceae	S/CSR	0	0	1	1
Holcus lanatus	Poaceae	CSR	1	0	1	0
Holcus mollis	Poaceae	C/CSR	1	0	0	1
Hypericum humifusum	Hypericaceae	SR/CSR	0	0	0	0
Hypochaeris radicata	Asteraceae	CSR	0	0	0	0
Jacobaea vulgaris	Asteraceae	R/CR	1	0	0	1
Juncus effusus	Juncaceae	C/SC	0	0	0	0
Lactuca serriola	Asteraceae	R	0	0	0	0
Lolium perenne	Poaceae	CR/CSR	0	0	0	0
Lotus corniculatus	Fabaceae	S/CSR	1	0	1	1
Luzula campestris	Cyperaceae	S/CSR	1	1	1	0
Malva moschata	Malvaceae	C/CSR	0	0	1	1
Medicago lupulina	Fabaceae	R/CSR	0	0	0	0
Papaver dubium	Papaveraceae	R	0	0	0	1
Phleum pratense subsp.bertolonii	Poaceae	CSR	0	0	0	0
Plantago lanceolata	Plantaginaceae	CSR	1	1	0	0
Poa annua	Poaceae	R	0	0	0	1
Poa pratensis	Poaceae	CSR	0	1	1	1
Poa trivialis	Poaceae	R/CSR	0	0	0	0
Potentilla erecta	Rosaceae	S/CSR	1	0	0	1
Prunella vulgaris	Lamiaceae	CSR	0	0	0	0
Prunus avium	Rosaceae	SC	0	0	0	0
Quercus cerris	Fagaceae	SC	0	0	1	0
Quercus robur	Fagaceae	SC	0	0	1	0
Ranunculus acris	Ranunculaceae	CSR	0	0	0	0
Ranunculus bulbosus	Ranunculaceae	SR	0	1	0	0
Ranunculus repens	Ranunculaceae	CR	1	0	1	1
Rubus sp	Rosaceae	SC	0	0	0	0
Rumex acetosa	Polygonaceae	CSR	1	1	0	0

Table S3.4 continued

Taxon	Family	C-S-R type	Rabbit resistant	Mollusc resistant	I+M resistant	Polyploid
Rumex acetosella	Polygonaceae	SR/CSR	1	0	1	1
Sambucus nigra	Adoxaceae	C	0	1	0	0
Sanguisorba minor	Rosaceae	S/CSR	0	0	0	1
Scorzoneroides autumnalis	Asteraceae	R/CSR	0	0	0	0
Sonchus asper	Asteraceae	R/CR	0	0	0	0
Stellaria graminea	Caryophyllaceae	CSR	1	0	0	0
Stellaria media	Caryophyllaceae	R	0	0	0	1
Taraxacum officinale	Asteraceae	R/CSR	0	0	0	0
Trifolium dubium	Fabaceae	R/SR	0	0	0	1
Trifolium pratense	Fabaceae	CSR	0	0	0	0
Trifolium repens	Fabaceae	CR/CSR	1	0	0	1
Trisetum flavescens	Poaceae	CSR	1	1	0	1
Urtica dioica	Urticaceae	C	0	0	0	1
Verbascum nigrum	Scrophulariaceae	C/CSR	0	0	0	0
Veronica arvensis	Plantaginaceae	SR	0	1	0	0
Veronica chamaedrys	Plantaginaceae	CSR	1	0	0	1
Veronica serpyllifolia	Plantaginaceae	R/CSR	0	0	0	0
Vicia sativa subsp.nigra	Fabaceae	R/CSR	0	1	1	0
Viola riviniana	Violaceae	S/CSR	0	1	0	1

Table S3.5 Correlations within control (herbivore-excluded plots) and plots with: **a**) rabbits, **b**) molluscs, **c**) insects and molluscs; between five plant community traits investigated in the path analyses: 1) BM: total biomass of each plot; 2) C: mean competition of each plot, estimated with phylogenetic GLS; 3) GS: biomass- weighted mean 1C-value (pg) of each plot, estimated with PGLS; 4) ploidy: percent biomass of polyploidy species on each plot; 5) HR: percent biomass of herbivore-resistant species on each plot. Number of plots = 62, 68, and 68 for insect, mollusc, and insect + molluscs respectively (including the controls).

a) Rabbits

Control - N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.098	-0.408	0.092	0.254	0.233
C	0.098	1	0.565	0.79	0.669	-0.634
Cval	-0.408	0.565	1	0.721	-0.018	-0.578
poly	0.092	0.79	0.721	1	0.228	-0.403
incr	0.254	0.669	-0.018	0.228	1	-0.59
forbs	0.233	-0.634	-0.578	-0.403	-0.59	1

Control + N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.098	-0.408	0.092	0.254	0.233
C	0.098	1	0.565	0.79	0.669	-0.634
Cval	-0.408	0.565	1	0.721	-0.018	-0.578
poly	0.092	0.79	0.721	1	0.228	-0.403
incr	0.254	0.669	-0.018	0.228	1	-0.59
forbs	0.233	-0.634	-0.578	-0.403	-0.59	1

+ Rabbits - N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.24	-0.51	-0.408	0.451	0.375
C	0.24	1	-0.426	-0.742	0.267	0.203
Cval	-0.51	-0.426	1	0.696	-0.884	-0.649
poly	-0.408	-0.742	0.696	1	-0.495	-0.116
incr	0.451	0.267	-0.884	-0.495	1	0.681
forbs	0.375	0.203	-0.649	-0.116	0.681	1

b) Molluscs

Control - N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.302	0.374	0.194	-0.408	-0.296
C	0.302	1	-0.49	-0.419	-0.721	-0.313
Cval	0.374	-0.49	1	0.8	0.589	-0.33
poly	0.194	-0.419	0.8	1	0.592	-0.145
incr	-0.408	-0.721	0.589	0.592	1	-0.018
forbs	-0.296	-0.313	-0.33	-0.145	-0.018	1

Control + N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.098	-0.408	0.092	-0.401	0.233
C	0.098	1	0.565	0.79	-0.444	-0.634
Cval	-0.408	0.565	1	0.721	0.27	-0.578
poly	0.092	0.79	0.721	1	-0.086	-0.403
incr	-0.401	-0.444	0.27	-0.086	1	0.224
forb	0.233	-0.634	-0.578	-0.403	0.224	1

+ Molluscs - N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.307	0.332	0.264	-0.051	-0.406
C	0.307	1	0.025	0.488	-0.552	-0.386
Cval	0.332	0.025	1	0.639	0.649	-0.729
poly	0.264	0.488	0.639	1	0.202	-0.548
incr	-0.051	-0.552	0.649	0.202	1	-0.035
forbs	-0.406	-0.386	-0.729	-0.548	-0.035	1

Table S3. 5 continued

a) Rabbits

+ Rabbits +N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.521	0.135	0.243	0.41	0.219
C	0.521	1	0.354	0.441	0.242	-0.19
Cval	0.135	0.354	1	0.793	-0.554	-0.542
poly	0.243	0.441	0.793	1	-0.247	-0.128
incr	0.41	0.242	-0.554	-0.247	1	0.393
forbs	0.219	-0.19	-0.542	-0.128	0.393	1

b) Molluscs

+ Molluscs + N

	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.652	-0.137	0.448	-0.486	0.596
C	0.652	1	0.136	0.433	-0.412	0.186
Cval	-0.137	0.136	1	0.597	0.764	-0.411
poly	0.448	0.433	0.597	1	0.283	0.219
incr	-0.486	-0.412	0.764	0.283	1	-0.408
forbs	0.596	0.186	-0.411	0.219	-0.408	1

c)	Insects + molluscs (IM)					
Control - N						
	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.302	0.374	0.194	-0.29	-0.296
C	0.302	1	-0.49	-0.419	-0.738	-0.313
Cval	0.374	-0.49	1	0.8	0.654	-0.33
poly	0.194	-0.419	0.8	1	0.541	-0.145
incr	-0.29	-0.738	0.654	0.541	1	-0.03
forbs	-0.296	-0.313	-0.33	-0.145	-0.03	1

Control + N						
	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.098	-0.408	0.092	-0.325	0.233
C	0.098	1	0.565	0.79	-0.935	-0.634
Cval	-0.408	0.565	1	0.721	-0.368	-0.578
poly	0.092	0.79	0.721	1	-0.756	-0.403
incr	-0.325	-0.935	-0.368	-0.756	1	0.485
forb	0.233	-0.634	-0.578	-0.403	0.485	1

+ IM - N						
	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.238	0.015	0.016	-0.212	0.028
C	0.238	1	0.605	0.451	0.134	-0.234
Cval	0.015	0.605	1	0.894	0.432	-0.497
poly	0.016	0.451	0.894	1	0.365	-0.432
incr	-0.212	0.134	0.432	0.365	1	-0.653
forbs	0.028	-0.234	-0.497	-0.432	-0.653	1

+ IM + N						
	BM	C	GS	Ploidy	HR	forbs
totBM	1	0.504	0.52	0.427	0.161	-0.419
C	0.504	1	0.902	0.829	0.091	-0.354
Cval	0.52	0.902	1	0.973	0.289	-0.316
poly	0.427	0.829	0.973	1	0.287	-0.293
incr	0.161	0.091	0.289	0.287	1	-0.002
forbs	-0.419	-0.354	-0.316	-0.293	-0.002	1

Table S3.6 Table showing conditional independence claims tested in the context of path analyses. Each number refers to a hypothetical directed acyclic path diagram (Fig S3.1). The d-*sep* path analysis method tests conditional independence between parameters in a path diagram. For example, the conditional independence between weighted mean GS and mean plot competition, given the experimental treatment (i.e. herbivory and nutrient input, which are the exogenous variables), can be drawn as GS ← treatment → competition, and written as: (GS, competition) | {treatment}). Independence between weighted mean GS and competition, while holding experimental treatment constant, is tested in a regression, where competition is a function of herbivory, nutrient treatment, and mean GS (i.e.: competition ~ treatment + GS). If the p-value for the coefficient of weighted mean GS is below the alpha level ($p \leq 0.05$), this indicates that GS and competition are not independent of each other given the experimental treatment.

Each conditional independence claim (set of parameters *not* connected by a path) in a path model is tested in this way to calculate its p-value). A variable may function simultaneously as a dependent variable (e.g. competition as a function of GS and the experimental treatments) and as predictor (e.g. competition as a predictor of plant resistance to grazing pressure). Fisher's C-statistic (Shipley 2002) is calculated from the p-values of the conditional independencies and the Chi-square distributed parameter *k*, which is equal to the number of conditional independencies in the model. The hypothetical path model is rejected when the C-statistic is below the alpha p-value, meaning that useful information is contained in one or more of the missing paths. Another goodness-of-fit statistic is the C-statistic Information Criterion (CICc) (von Hardenberg & Gonzalez-Voyer 2013; Gonzalez-Voyer & von Hardenberg 2014) which also takes into account sample size and the number of parameters in the model.

The experiment treatments were fitted as 2 x 2 x 2 (N x P x herbivore where each is a binary factor) in the model equations. In terms of the regression coefficients and p-values returned for each community property, this is equivalent to scoring the experiment as a single factor variable with eight levels; however with additional information on their interactions. We thus have a total of six parameters: the experiment, weighted mean GS, polyploid abundance, grazing resistance, mean competition, total biomass).

Shown below are the p-values obtained for each conditional independence claim, which were tested with generalised least squares (GLS) with ten different variance structures (varID): 1) plot; 2) N; 3) P; 4) herbivore type; 5) N + herbivore type; 6) N + P; 7) plot * N; 8) herbivore type * N; 9) herbivore type * plot * N; 10) herbivore type * P * N; and, 11) no variance structure. If the varID column is left blank, no variance structures were used. The p-value and CIC (C-statistic information criterion) for the structural model are also shown. A

significant p-value indicates there are one or more missing paths in the path diagram; the C-statistic and CICc indicate goodness of fit, a lower value is preferable. BM= total plot biomass, RR= percent rabbit-resistant biomass of each plot; MR = mollusc-resistant; IMR = insect + mollusc-resistant biomass of each plot; GS= biomass-weighted mean GS of each plot, C = mean weighted competition of each plot also estimated by PGLS, ploidy = percent biomass of polyploid species on each plot; exp = experimental treatment, i.e.: herbivore * N * P.

Table S3.6 a) Rabbits:

			Rabbits				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
1	(ploidy, BM) | exp, C, GS	BM ~ exp + C + GS + ploidy	0.1650				
	(GS, BM) | exp, C	BM ~ exp + C + GS	0.1104				
	(RR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + RR	0.3387		10.18	0.1174	62.083
2	(ploidy, BM) | exp, C	BM ~ exp + C + ploidy	0.8380				
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.0363				
	(RR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + RR	0.3387		9.15	0.1653	61.058
3	(ploidy, BM) | exp, C, RR, GS	BM ~ exp + C + RR + GS + ploidy	0.2733				
	(GS, BM) | exp, C, RR	BM ~ exp + C + RR + GS	0.5948		3.63	0.4579	59.729
4	(RR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + RR	0.3387				
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.0363		8.80	0.0664	64.892
5	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1384				
	(C, BM) | exp, ploidy, GS, RR	BM ~ exp + ploidy + GS + RR + C	0.2400		6.81	0.1463	62.905
6	(GS, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + GS	0.3216				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1384				
	(C, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + C	0.3101		8.47	0.3887	56.382
7	(GS, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + GS	0.3216				

Table S3.6 a) continued

No.	Conditional independence claim	Claim test	Rabbits				
			GLS p-value	varID	C-stat	p-value	CICc
	(C, BM) | exp, ploidy, GS, RR	BM ~ exp + ploidy + GS + RR + C	0.2400		5.12	0.2749	61.218
8	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1384				
	(GS, BM) | exp, RR	BM ~ exp + RR + GS	0.8782				
	(C, BM) | exp, ploidy, GS, RR	BM ~ exp + ploidy + GS + RR + C	0.2400		6.72	0.3476	58.626
9	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(GS, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + GS	0.3216				
	(C, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + C	0.3101		4.87	0.5609	56.775
10	(GS, BM) | exp, RR	BM ~ exp + RR + GS	0.8782				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1384				
	(C, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + C	0.1384		6.46	0.5955	54.372
11	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(C, BM) | exp, ploidy, GS, RR	BM ~ exp + ploidy + GS + RR + C	0.2400		3.11	0.5394	59.2065
12	(ploidy, BM) | exp, C, RR	BM ~ exp + C + RR + ploidy	0.7018				
	(exp, GS) | ploidy, RR, C, BM	GS ~ ploidy + RR + C + BM + exp	0.0043		11.61	0.0205	67.702
13	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1649	-			
	(ploidy, RR) | exp, GS, C	RR ~ exp + GS + C + ploidy	0.0622				
	(GS, BM) | exp, RR	BM ~ exp + RR + GS	0.8782				
	(C, BM) | exp, ploidy, GS, RR	BM ~ exp + ploidy + GS + RR + C	0.2400		12.62	0.1255	60.533
14	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(GS, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + GS	0.3216				
	(ploidy, RR) | exp, GS, C	RR ~ exp + GS + C + ploidy	0.0622				

Table S3.6 a) continued

No.	Conditional independence claim	Claim test	Rabbits GLS p-value	varID	C-stat	p-value	CICc
	(C, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + C	0.3101		10.42	0.2366	58.332
15	(GS, BM) | exp, RR	BM ~ exp + RR + GS	0.8782				
	(ploidy, BM) | exp, RR, GS	BM ~ exp + RR + GS + ploidy	0.1384				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(C, BM) | exp, ploidy, RR	BM ~ exp + ploidy + RR + C	0.3101				
	(ploidy, RR) | exp, GS, C	RR ~ exp + GS + C + ploidy	0.0622		12.02	0.2838	56.107
16	(exp, BM) | ploidy, C, RR	BM ~ ploidy + C + RR + exp	0.0003				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.8794				
	(ploidy, RR) | exp, GS, C	RR ~ exp + GS + C + ploidy	0.0622				
	(GS, BM) | exp, C, RR, ploidy	BM ~ exp + C + RR + ploidy + GS	0.2482		24.82	0.0017	72.731
17	(ploidy, C) | exp, GS	C ~ exp + GS + ploidy	0.0081	block			
	(ploidy, RR) | exp, GS, C	RR ~ exp + GS + C + ploidy	0.0622				
	(ploidy, BM) | exp, C, RR, GS	BM ~ exp + C + RR + GS + ploidy	0.2733				
	(GS, C) | exp	C ~ exp + GS	0.0026	block			
	(GS, RR) | exp, C	RR ~ exp + C + GS	0.0000				
	(GS, BM) | exp, C, RR	BM ~ exp + C + RR + GS	0.5948		53.75	0.0000	97.839

Table S3.6 b) Molluscs:

			Molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
1	(ploidy, BM) | exp, C, GS	BM ~ exp + C + GS + ploidy	0.0688				
	(GS, BM) | exp, C	BM ~ exp + C + GS	0.6072				
	(MR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + MR	0.0038		17.50	0.0076	67.456
2	(ploidy, BM) | exp, C	BM ~ exp + C + ploidy	0.3075				
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.1105				

Table S3.6 b) continued

			Molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
	(MR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + MR	0.0038		17.91	0.0065	67.869
3	(ploidy, BM) | exp, C, MR, GS	BM ~ exp + C + MR + GS + ploidy	0.0293	N			
	(GS, BM) | exp, C, MR	BM ~ exp + C + MR + GS	0.0031	N	18.61	0.0009	72.446
4	(MR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + MR	0.0038				
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.1105		15.55	0.0037	69.384
5	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(C, BM) | exp, ploidy, GS, MR	BM ~ exp + ploidy + GS + MR + C	0.4342		8.16	0.0860	61.9902
6	(GS, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + GS	0.8584				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(C, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + C	0.4805		11.22	0.1895	57.462
7	(GS, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + GS	0.8584				
	(C, BM) | exp, ploidy, GS, MR	BM ~ exp + ploidy + GS + MR + C	0.4342		1.97	0.7406	55.8072
8	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(GS, BM) | exp, MR	BM ~ exp + MR + GS	0.0010	N			

Table S3.6 b) continued

			Molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
	(C, BM) | exp, ploidy, GS, MR	BM ~ exp + ploidy + GS + MR + C	0.4342		21.97	0.0012	71.932
9	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0390				
	(GS, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + GS	0.0010	N			
	(C, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + C	0.4342		21.97	0.0012	71.932
10	(GS, BM) | exp, MR	BM ~ exp + MR + GS	0.0010	N			
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(C, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + C	0.4805		24.73	0.0017	70.972
11	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(C, BM) | exp, ploidy, GS, MR	BM ~ exp + ploidy + GS + MR + C	0.4342		4.63	0.3273	58.4639
12	(ploidy, BM) | exp, C, MR	BM ~ exp + C + MR + ploidy	0.0073				
	(exp, GS) | ploidy, MR, C, BM	GS ~ ploidy + MR + C + BM + exp	0.4761	block	11.32	0.0232	65.157
13	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(ploidy, MR) | exp, GS, C	MR ~ exp + GS + C + ploidy	0.3341	block+N			
	(GS, BM) | exp, MR	BM ~ exp + MR + GS	0.0010	N			
	(C, BM) | exp, ploidy, GS, MR	BM ~ exp + ploidy + GS + MR + C	0.4342		24.17	0.0072	66.832
14	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(GS, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + GS	0.8584				
	(ploidy, MR) | exp, GS, C	MR ~ exp + GS + C + ploidy	0.3341	block+N			
	(C, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + C	0.4805		6.93	0.5446	53.166
15	(GS, BM) | exp, MR	BM ~ exp + MR + GS	0.0010	N			
	(ploidy, BM) | exp, MR, GS	BM ~ exp + MR + GS + ploidy	0.0390				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(C, BM) | exp, ploidy, MR	BM ~ exp + ploidy + MR + C	0.4805				

Table S3.6 b) continued

			Molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
	(ploidy, MR) | exp, GS, C	MR ~ exp + GS + C + ploidy	0.3341	block+N	26.92	0.0027	69.591
16	(exp, BM) | ploidy, C, MR	BM ~ ploidy + C + MR + exp	0.2094				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.2274				
	(ploidy, MR) | exp, GS, C	MR ~ exp + GS + C + ploidy	0.3341	block+N			
	(GS, BM) | exp, C, MR, ploidy	BM ~ exp + C + MR + ploidy + GS	0.6949		9.01	0.3415	55.2497
17	(ploidy, C) | exp, GS	C ~ exp + GS + ploidy	0.0000				
	(ploidy, MR) | exp, GS, C	MR ~ exp + GS + C + ploidy	0.3341	block+N			
	(ploidy, BM) | exp, C, MR, GS	BM ~ exp + C + MR + GS + ploidy	0.0293	N			
	(GS, C) | exp	C ~ exp + GS	0.0314				
	(GS, MR) | exp, C	MR ~ exp + C + GS	0.0000	block+N			
	(GS, BM) | exp, C, MR	BM ~ exp + C + MR + GS	0.0031	N	73.78	0.0000	116.446

Table S3.6 c) Insects + molluscs (IM)

			Insects & molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
1	(ploidy, BM) | exp, C, GS	BM ~ exp + C + GS + ploidy	0.3564	N+IM			
	(GS, BM) | exp, C	BM ~ exp + C + GS	0.9677	N+IM			
	(IMR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + IMR	0.5516	N+IM	3.32	0.7679	53.278
2	(ploidy, BM) | exp, C	BM ~ exp + C + ploidy	0.5996	N+IM			
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.4415	N+IM			
	(IMR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + IMR	0.5516	N+IM	3.85	0.6972	53.807
3	(ploidy, BM) | exp, C, IMR, GS	BM ~ exp + C + IMR + GS + ploidy	0.6203	N+IM			
	(GS, BM) | exp, C, IMR	BM ~ exp + C + IMR + GS	0.7116	N+IM	1.64	0.8024	55.469
4	(IMR, BM) | exp, ploidy, GS, C	BM ~ exp + ploidy + GS + C + IMR	0.5516	N+IM			
	(GS, BM) | exp, ploidy, C	BM ~ exp + ploidy + C + GS	0.4415	N+IM	2.83	0.5875	56.658
5	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(C, BM) | exp, ploidy, GS, IMR	BM ~ exp + ploidy + GS + IMR + C	0.2377	N+IM	3.15	0.5335	56.9808
6	(GS, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + GS	0.5257	N+IM			
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(C, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + C	0.1822	N+IM	10.83	0.2114	57.073
7	(GS, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + GS	0.5257	N+IM			
	(C, BM) | exp, ploidy, GS, IMR	BM ~ exp + ploidy + GS + IMR + C	0.2377	N+IM	4.16	0.3848	57.9929
8	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(GS, BM) | exp, IMR	BM ~ exp + IMR + GS	0.0778	N+IM			
	(C, BM) | exp, ploidy, GS, IMR	BM ~ exp + ploidy + GS + IMR + C	0.2377	N+IM	8.25	0.2200	58.214

Table S3.6 c) continued

No.	Conditional independence claim	Claim test	Insects & molluscs GLS p-value	varID	C-stat	p-value	CICc
9	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(GS, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + GS	0.5257	N+IM			
	(C, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + C	0.1822	N+IM	10.56	0.1030	60.518
10	(GS, BM) | exp, IMR	BM ~ exp + IMR + GS	0.0778	N+IM			
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(C, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + C	0.1822	N+IM	14.65	0.0662	60.894
11	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(C, BM) | exp, ploidy, GS, IMR	BM ~ exp + ploidy + GS + IMR + C	0.2377	N+IM	8.74	0.0679	62.5742
12	(ploidy, BM) | exp, C, IMR	BM ~ exp + C + IMR + ploidy	0.5664	N+IM			
	(exp, GS) | ploidy, IMR, C, BM	GS ~ ploidy + IMR + C + BM + exp	0.0077	Block*IM*N	10.87	0.0281	64.703
13	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(ploidy, IMR) | exp, GS, C	IMR ~ exp + GS + C + ploidy	0.0000	IM			
	(GS, BM) | exp, IMR	BM ~ exp + IMR + GS	0.0778	N+IM			
	(C, BM) | exp, ploidy, GS, IMR	BM ~ exp + ploidy + GS + IMR + C	0.2377	N+IM	31.28	0.0001	77.521
14	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(GS, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + GS	0.5257	N+IM			
	(ploidy, IMR) | exp, GS, C	IMR ~ exp + GS + C + ploidy	0.0000	IM			
	(C, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + C	0.1822	N+IM	33.58	0.0000	79.825
15	(GS, BM) | exp, IMR	BM ~ exp + IMR + GS	0.0778	N+IM			
	(ploidy, BM) | exp, IMR, GS	BM ~ exp + IMR + GS + ploidy	0.8720	N+IM			
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(C, BM) | exp, ploidy, IMR	BM ~ exp + ploidy + IMR + C	0.1822	N+IM			

Table S3.6 c) continued

			Insects & molluscs				
No.	Conditional independence claim	Claim test	GLS p-value	varID	C-stat	p-value	CICc
	(ploidy, IMR) | exp, GS, C	IMR ~ exp + GS + C + ploidy	0.0000	IM	37.68	0.0000	80.346
16	(exp, BM) | ploidy, C, IMR	BM ~ ploidy + C + IMR + exp	0.0001				
	(GS, C) | exp, ploidy	C ~ exp + ploidy + GS	0.0532				
	(ploidy, IMR) | exp, GS, C	IMR ~ exp + GS + C + ploidy	0.0000	IM			
	(GS, BM) | exp, C, IMR, ploidy	BM ~ exp + C + IMR + ploidy + GS	0.8140	N+IM	47.73	0.0000	95.6346
17	(ploidy, C) | exp, GS	C ~ exp + GS + ploidy	0.0361				
	(ploidy, IMR) | exp, GS, C	IMR ~ exp + GS + C + ploidy	0.0000	IM			
	(ploidy, BM) | exp, C, IMR, GS	BM ~ exp + C + IMR + GS + ploidy	0.6203	N+IM			
	(GS, C) | exp	C ~ exp + GS	0.0000	IM			
	(GS, IMR) | exp, C	IMR ~ exp + C + GS	0.1758	IM*N*P			
	(GS, BM) | exp, C, IMR	BM ~ exp + C + IMR + GS	0.7116	N+IM	57.81	0.0000	100.474

Table S3.7 Summary path model goodness-of-fit statistics: Fisher's C-statistic and CICc (C-statistic information criterion) and p-values. P-values above the alpha value (0.05) indicate the conditional independencies are satisfied and the model is a plausible model.)

	Rabbits			Molluscs			Insects & molluscs		
Model	C-stat	p-value	CICc	C-stat	p-value	CICc	C-stat	p-value	CICc
1	10.1762	0.1174	62.083	17.4964	0.0076	67.456	3.3189	0.7679	53.278
2	9.1506	0.1653	61.058	17.9096	0.0065	67.869	3.8480	0.6972	53.807
3	3.6334	0.4579	59.729	18.6130	0.0009	72.446	1.6356	0.8024	55.469
4	8.7972	0.0664	64.892	15.5510	0.0037	69.384	2.8250	0.5875	56.658
5	6.8094	0.1463	62.905	8.1569	0.0860	61.9902	3.1474	0.5335	56.9808
6	8.4725	0.3887	56.382	11.2217	0.1895	57.462	10.8327	0.2114	57.073
7	5.1231	0.2749	61.218	1.9739	0.7406	55.8072	4.1595	0.3848	57.9929
8	6.7188	0.3476	58.626	21.9724	0.0012	71.932	8.2547	0.2200	58.214
9	4.8676	0.5609	56.775	21.9724	0.0012	71.932	10.5587	0.1030	60.518
10	6.4633	0.5955	54.372	24.7318	0.0017	70.972	14.6539	0.0662	60.894
11	3.1113	0.5394	59.2065	4.6306	0.3273	58.4639	8.7409	0.0679	62.5742
12	11.6065	0.0205	67.702	11.3240	0.0232	65.157	10.8700	0.0281	64.703
13	12.6240	0.1255	60.533	24.165	0.0072	66.832	31.281	0.0001	77.521
14	10.4225	0.2366	58.332	6.9259	0.5446	53.166	33.5846	0.0000	79.825
15	12.0182	0.2838	56.107	26.9245	0.0027	69.591	37.6797	0.0000	80.346
16	24.8223	0.0017	72.731	9.0097	0.3415	55.2497	47.7255	0.0000	95.6346
17	53.7504	0.0000	97.839	73.7793	0.0000	116.446	57.8070	0.0000	100.474

Table S3.8 a) MCMCglmm output in which plant biomass was fitted as a function of genome size (C-value), ploidy, and the herbivore and nutrient experimental treatment. Insect, rabbit and mollusc inclusion/exclusion, N, P, and K are scored as +/- binary factors. Baseline levels are exclusion/minus in each experimental treatment, and diploid for the ploidy parameter. Significant parameters (pMCMC < 0.05) are highlighted in bold. **b**) Random and residual effects.

a)

	Posterior mean	95% Confidence intervals	Effective sample size	pMCMC
Intercept	0.232	-3.73, 4.40	9705	0.9122
Ploidy	-0.985	-2.69, 0.69	9980	0.2491
Insects	0.160	-0.34, 0.68	9980	0.5104
C-value	0.084	-0.20, 0.38	9980	0.5503
Rabbits	0.159	-0.21, 0.57	10263	0.4239
Mollluscs	-0.205	-0.74, 0.37	9980	0.4321
N	**0.522**	**0.13, 0.90**	**9980**	**0.0078**
P	**0.128**	**0.02, 0.24**	**10336**	**0.0273**
K	0.084	-0.03, 0.20	9980	0.1451
Ploidy : insects	**-0.344**	**-0.59, -0.10**	**9980**	**0.0038**
Insects : C-value	-0.029	-0.07, 0.01	9980	0.1762
Insects : rabbits	**-0.371**	**-0.73, -0.02**	**9980**	**0.0451**
Insects : mollluscs	-0.123	-0.86, 0.53	9980	0.6928
Rabbits : mollluscs	0.264	-0.12, 0.65	9980	0.1792
Ploidy : C-value	**0.502**	**0.09, 0.90**	**9980**	**0.0170**
C-value : mollluscs	-0.003	-0.08, 0.08	10396	0.9453
Ploidy : mollluscs	-0.287	-0.72, 0.13	10770	0.1880
C-value : N	-0.017	-0.13, 0.10	9980	0.7842
Ploidy : N	0.017	-0.64, 0.63	9980	0.9551
Mollluscs : N	-0.138	-0.50, 0.22	10418	0.4475
C-value : rabbits	-0.001	-0.09, 0.08	9980	0.9870
Ploidy : rabbits	**0.505**	**0.04, 0.98**	**9774**	**0.0355**
Rabbits : N	0.211	-0.19, 0.60	9980	0.3038
Insects : rabbits : mollluscs	0.053	-0.41, 0.55	10300	0.8309
Ploidy : C-value : mollluscs	**0.128**	**0.02, 0.24**	**9980**	**0.0220**
Ploidy : C-value : N	0.052	-0.12, 0.21	9980	0.5395
C-value : mollluscs : N	0.004	-0.11, 0.11	9980	0.9443
Ploidy : mollluscs : N	-0.386	-1.00, 0.22	9980	0.2144
Ploidy : C-value : rabbits	**-0.355**	**-0.48, -0.24**	**9980**	**< 0.0001**
C-value : rabbits : N	-0.008	-0.13, 0.10	9980	0.8940
Ploidy : rabbits : N	-0.086	-0.73, 0.58	9980	0.7990
Ploidy : C-value : mollluscs : N	**0.205**	**0.05, 0.36**	**9980**	**0.0084**
Ploidy : C-value : rabbits : N	**-0.278**	**-0.45, -0.11**	**9980**	**0.0024**

Table S3.8 b)

G-structure: random effects

	Posterior mean	95% Confidence intervals	Effective sample size
Plot	0.043	0, 0.13	10284
Phylogeny	22.110	10.23, 33.92	9980
Species	0.481	0, 2.44	9980

R-structure: residual structure

	Posterior mean	95% Confidence intervals	Effective sample size
Insects + molluscs	31.658	30.27, 33.12	9980
All	13.830	13.23, 14.45	9980
Insects	37.381	35.67, 39	9980
Insects + rabbits	9.882	9.41, 10.32	9980
Molluscs	41.485	39.68, 43.38	9980
Molluscs + rabbits	30.277	28.95, 31.6	9980
Control	48.550	46.35, 50.74	9980
Rabbits	29.275	27.89, 30.72	9980

Table S3.9 Step-wise model reduction with AIC and p-values of each assessed parameter in testing PGLS biomass-weighted genome size.

Fixed effects	Difference in AIC	p-value
Full model	2084.6	NA
K	-2	0.8275
Mg	-2	0.8326
Rabbits : molluscs : insects : N : P	1.8	0.05
Rabbits: molluscs : insects : N	-2	0.9233
Rabbits: molluscs : insects : P	-1.8	0.6538
Rabbits: molluscs : N : P	-1.6	0.5348
Rabbits: insects : N : P	-1.4	0.4482
Molluscs : insects : N : P	-1.8	0.6744
Rabbits: molluscs : insects	-0.5	0.2219
Rabbits: insects : N	-1.4	0.418
Rabbits: molluscs : P	-2	0.9807
Rabbits: insects : P	-2	0.8972
Molluscs : insects : P	-0.6	0.2357
Rabbits: N : P	-0.9	0.29
Molluscs : N : P	-0.5	0.2179
Insects : N : P	-1.4	0.4491
Rabbits: insects	-2	0.9117
Rabbits: P	0	0.1561
Molluscs : P	0	0.1531
Insects : P	-2	0.9015
N : P	-1.5	0.4942
Rabbits: molluscs : N	1.7	0.0572
Rabbits: molluscs	-2	0.8732

Table S3.10 Effects of experimental treatment (herbivore exclusion and nitrogen (N) and phosphate (P) input) on biomass-weighted mean GS. The most parsimonious LME model shows a significant increase in mean GS with a three-way effect of N, molluscs, and insects; conversely the combined effect of nitrogen and rabbit grazing shows a decrease in mean GS. Significant terms ($p < 0.05$) are highlighted in bold.

	B	Std. error	t-value	p-value	DF	F-value	p-value
(Intercept)	5.70	0.41	13.89	< 0.0001	1, 541	668.54	< 0.0001
Rabbits	**-1.36**	**0.18**	**-7.56**	**< 0.0001**	**1, 541**	**161.48**	**< 0.0001**
N	**-0.99**	**0.30**	**-3.36**	**0.0008**	**1, 541**	**161.33**	**< 0.0001**
Molluscs	1.02	0.56	1.84	0.1400	1, 4	7.15	0.0556
Insects	0.57	0.56	1.03	0.3617	1, 4	0.44	0.5453
P	**-0.32**	**0.13**	**-2.52**	**0.0119**	**1, 541**	**6.62**	**0.0103**
N: rabbits	**-0.52**	**0.25**	**-2.06**	**0.0403**	**1, 541**	**4.35**	**0.0374**
Molluscs : insects	-0.35	0.78	-0.44	0.6813	1, 4	0.19	0.6833
N : molluscs	-0.40	0.37	-1.08	0.2820	1, 541	1.53	0.2173
N : insects	**-0.99**	**0.37**	**-2.69**	**0.0074**	**1, 541**	**1.29**	**0.2559**
N : molluscs : insects	**1.35**	**0.51**	**2.64**	**0.0086**	**1, 541**	**6.97**	**0.0086**

Table S3.11 a) Means and standard deviations of biomass-weighted mean GS and total biomass of plots, shown for each of eight herbivore treatments and +/- nitrogen (N) treatment. Total number of plots= 556. **b**) Means and standard deviations are shown for the plots included in the path analyses. (Ins = insects, mol= molluscs, control = all herbivores excluded.

a)

Herbivore treatment	N	Weighted mean GS	Total biomass (g)	Number of species	Number of plots
control	-	5.79 ± 1.26	57.58 ± 15.11	9.48 ± 2.49	33
control	+	4.43 ± 1.40	100.83 ± 26.24	5.88 ± 2.01	32
+ ins	-	6.15 ± 1.42	65.79 ± 15.97	8.33 ± 3.03	36
+ ins	+	3.93 ± 1.45	83.85 ± 23.41	6.08 ± 2.37	36
+ mol	-	6.40 ± 1.19	52.93 ± 17.30	6.44 ± 1.66	36
+ mol	+	5.19 ± 1.80	90.30 ± 30.56	4.81 ± 1.39	36
+ rabbits	-	3.90 ± 1.32	48.72 ± 18.59	10.77 ± 2.06	30
+ rabbits	+	2.82 ± 1.29	74.63 ± 31.13	8.48 ± 2.26	29
+ ins + mol	-	6.68 ± 1.42	43.29 ± 11.23	7.11 ± 2.08	36
+ ins + mol	+	6.05 ± 2.35	76.92 ± 21.44	4.89 ± 1.86	36
+ ins + rabbits	-	4.72 ± 1.53	29.79 ± 10.34	8.94 ± 3.07	36
+ ins + rabbits	+	2.44 ± 1.03	43.61 ± 16.70	8.28 ± 2.79	36
+ mol + rabbits	-	5.36 ± 1.67	59.79 ± 23.72	8.28 ± 2.77	36
+ mol + rabbits	+	3.27 ± 1.72	78.23 ± 31.46	7.14 ± 2.51	36
+ ins + mol + rabbits	-	5.54 ± 1.40	33.14 ± 15.17	9.33 ± 2.33	36
+ ins + mol + rabbits	+	3.58 ± 1.90	46.40 ± 24.39	8.44 ± 3.01	36

b)

Herbivore treatment	N	Weighted mean GS	Total biomass (g)	Number of species	Number of plots
control	-	6.07 ± 0.91	55.06 ± 18.82	9.94 ± 2.43	16
control	+	4.49 ± 1.47	94.01 ± 21.4	6.81 ± 1.72	16
+ mol	-	6.14 ± 1.28	48.12 ± 17.39	6.83 ± 1.82	18
+ mol	+	5.62 ± 1.69	84.6 ± 27.05	4.78 ± 1.59	18
+ rabbits	-	3.82 ± 1.18	47.03 ± 19.21	11.07 ± 2.02	15
+ rabbits	+	2.78 ± 1.55	75.92 ± 33.11	9.4 ± 2.38	15
+ ins + mol	-	6.79 ± 1.25	39.8 ± 10.08	6.83 ± 2.04	18
+ ins + mol	+	6.12 ± 2.37	73.72 ± 17.87	5.22 ± 1.59	18

Table S3.12. **a**) Total biomass as a function of herbivore and nutrient treatments, in a linear mixed effect model. The most parsimonious model output is shown, following model reduction methods as described in the text. **b**) Species number as a function of herbivore and nutrient treatments, regressed in a generalised linear model with a Poisson error distribution and log link. The most parsimonious model is shown, as in **a**).

a)

	LME				ANOVA		
	B	Std.Error	t-value	p-value	DF	F-value	p-value
(Intercept)	51.91	6.21	8.36	< 0.0001	1, 538	493.98	< 0.0001
Rabbits	-10.52	3.55	-2.97	0.0031	1, 538	129.94	< 0.0001
Molluscs	-4.79	8.42	-0.57	0.5993	1, 4	0.27	0.6319
Insects	4.4	8.42	0.52	0.629	1, 4	9.94	0.0344
N	42.9	4.01	10.7	< 0.0001	1, 538	214.05	< 0.0001
P	8.21	1.83	4.49	< 0.0001	1, 538	30.44	< 0.0001
K	4.54	1.83	2.49	0.0132	1, 538	6.22	0.0129
Rabbits : molluscs	16.27	3.46	4.71	< 0.0001	1, 538	24.08	< 0.0001
Rabbits : insects	-19.25	3.46	-5.57	< 0.0001	1, 538	31.17	< 0.0001
Molluscs : insects	-12.92	11.63	-1.11	0.3288	1, 4	0.25	0.6433
Rabbits : N	-15.19	3.45	-4.4	< 0.0001	1, 538	19.92	< 0.0001
Molluscs : N	-7.4	4.99	-1.48	0.1386	1, 538	0.05	0.8196
Insects : N	-19.37	4.99	-3.88	0.0001	1, 538	11.3	0.0008
Molluscs : insects : N	14.91	6.92	2.15	0.0316	1, 538	4.64	0.0316

b)

	GLM				ANOVA		
Fixed effects	B	Std. Error	z-value	p-value	Deviance residuals	DF	Residual deviance
(Intercept)	2.23	0.05	45.541	< 0.0001			610.98
Insects	-0.09	0.04	-2.051	0.0403	0.191	554	610.79
N	-0.29	0.06	-5.155	< 0.0001	60.02	553	550.77
P	0.08	0.04	1.864	0.0623	0.621	552	550.15
Rabbits	0.10	0.05	1.99	0.0466	77.433	551	472.71
Molluscs	-0.35	0.06	-6.145	< 0.0001	26.271	550	446.44
K	-0.11	0.03	-3.33	0.0009	11.14	549	435.3
N : P	-0.16	0.06	-2.567	0.0103	6.886	548	428.42
Rabbits : N	0.23	0.06	3.664	0.0002	13.532	547	414.88
Rabbits : molluscs	0.16	0.06	2.503	0.0123	6.46	546	408.42
Insects : molluscs	0.20	0.06	3.212	0.0013	10.318	545	398.11

Table S3.13 Regression output and variance structures (var), showing the partial regression coefficients of path models in Fig. 3.2 (rabbits : table (**i**) below); and Fig 3.3 (molluscs, table (**ii**) below). Also shown in table (**iii**) is the regression output for molluscs & insects (Fig S3.3). Var = na indicates that the regression was fitted without a variance structure.

i) Rabbits

a) Genome size, var = N

	B	Std.Error	t-value	p-value
Intercept	6.42	0.33	19.26	< 0.0001
rab	-2.12	0.45	-4.71	< 0.0001
N	-1.76	0.57	-3.08	0.0032
P	-0.29	0.51	-0.58	0.5668
Plot	-0.53	0.31	-1.74	0.0883
Rabbits : N	0.27	0.83	0.32	0.7499
Rabbits : P	-0.38	0.72	-0.52	0.6042
N : P	0.52	1.00	0.52	0.6018
Rabbits : N : P	0.74	1.42	0.52	0.6045

b) Competition, var= plot

	B	Std.Error	t-value	p-value
Intercept	-0.06	0.09	-0.60	0.5501
Rabbits	0.01	0.05	0.16	0.8754
N	0.14	0.05	2.91	0.0052
P	0.12	0.05	2.29	0.0259
Ploidy	0.00	0.00	4.36	0.0001
Plot	0.14	0.04	3.76	0.0004
Rabbits : N	-0.03	0.06	-0.41	0.6869
Rabbits : P	-0.04	0.08	-0.55	0.5827
N : P	0.02	0.08	0.20	0.8443
Rabbits : N : P	0.06	0.11	0.52	0.6055

c) Polyploidy, var = N + rabbits

	B	Std.Error	t-value	p-value
Intercept	48.77	6.70	7.28	< 0.0001
Rabbits	7.53	3.63	2.08	0.0429
N	-0.51	4.47	-0.11	0.9105
P	-2.84	2.64	-1.07	0.2880
C-value	7.19	1.00	7.17	< 0.0001

Table S3.13 c) continued

	B	Std.Error	t-value	p-value
Plot	-7.01	2.08	-3.37	0.0014
Rabbits : N	-14.67	7.50	-1.96	0.0556
Rabbits : P	0.09	4.70	0.02	0.9842
N : P	7.51	7.28	1.03	0.3073
Rabbits : N : P	-5.46	12.96	-0.42	0.6752

d) Rabbit-resistant, var = na

	B	Std.Error	t-value	p-value
Intercept	40.16	13.41	3.00	0.0043
Rabbits	5.02	8.27	0.61	0.5465
N	11.15	7.69	1.45	0.1534
P	14.95	8.20	1.82	0.0743
C-value	-12.47	2.42	-5.15	< 0.0001
Competition	51.97	16.98	3.06	0.0035
Ploidy	0.40	0.21	1.91	0.0622
Plot	4.77	4.83	0.99	0.3283
Rabbits : N	14.63	10.34	1.42	0.1632
Rabbits : P	-7.76	11.54	-0.67	0.5044
N : P	4.11	11.77	0.35	0.7285
Rabbits : N : P	-0.05	16.82	0.00	0.9979

e) Biomass, var = na

	B	Std.Error	t-value	p-value
Intercept	45.49	7.65	5.95	< 0.0001
Rabbits	-11.52	11.02	-1.05	0.3007
N	30.84	10.77	2.86	0.0060
P	1.79	11.78	0.15	0.8800
Rabbit-resistant	0.38	0.15	2.54	0.0141
Plot	-2.88	5.95	-0.48	0.6312
Rabbits : N	-29.42	13.99	-2.10	0.0403
Rabbits : P	-16.80	16.30	-1.03	0.3076
N : P	-13.36	16.52	-0.81	0.4221
Rabbits : N : P	55.32	23.51	2.35	0.0224

Table S3.13 ii) Molluscs

a) Genome size, var = na

	B	Std.Error	t-value	p-value
Intercept	6.20	0.41	15.17	0
Mollusc	-0.07	0.55	-0.12	0.9046
N	-1.79	0.56	-3.17	0.0024
P	-0.35	0.67	-0.52	0.607
Mollusc : N	2.02	0.77	2.61	0.0113
Mollusc : P	0.35	0.93	0.37	0.7096
N : P	0.61	0.97	0.63	0.5327
Mollusc : N : P	-2.84	1.33	-2.14	0.0368

b) Competition, var = na

	B	Std.Error	t-value	p-value
Intercept	-0.09	0.09	-0.94	0.3511
Mollusc	-0.01	0.04	-0.18	0.8591
N	0.16	0.05	3.50	0.0009
P	0.10	0.05	1.93	0.059
Polyploidy	0.00	0.00	5.01	0
Mollusc : N	-0.12	0.06	-1.97	0.053
Mollusc : P	-0.09	0.07	-1.18	0.241
N : P	-0.01	0.08	-0.11	0.9129
Mollusc : N : P	0.06	0.10	0.59	0.56

c) Polyploidy, var = N

	B	Std.Error	t-value	p-value
Intercept	54.11	5.44	9.95	0
Mollusc	1.59	2.58	0.62	0.5408
N	-3.24	4.47	-0.72	0.4718
P	-3.99	3.12	-1.28	0.206
piCval	5.87	0.82	7.15	0
Mollusc : N	-0.94	6.05	-0.15	0.8774
Mollusc : P	-1.07	4.34	-0.25	0.806
N : P	9.39	7.44	1.26	0.2121
Mollusc : N : P	5.12	10.43	0.49	0.6255

Table S3.13 continued

d) Mollusc-resistant, var = na

	B	Std.Error	t-value	p-value
Intercept	65.22	7.40	8.81	0
Mollusc	2.50	3.07	0.82	0.4183
N	-14.88	5.33	-2.79	0.0071
P	-8.15	4.36	-1.87	0.0663
piCval	8.56	1.03	8.35	0
piC	-92.68	11.31	-8.20	0
Mollusc : N	17.66	6.49	2.72	0.0085
Mollusc : P	8.22	5.40	1.52	0.1334
N : P	-6.26	8.36	-0.75	0.4574
Mollusc : N : P	-6.05	10.83	-0.56	0.5785

e) Biomass, var= na

	B	Std.Error	t-value	p-value
Intercept	43.19	19.94	2.17	0.0345
Mollusc	-2.06	8.15	-0.25	0.8014
N	31.60	9.86	3.21	0.0022
P	3.63	10.13	0.36	0.7215
Mollusc- resistant	-0.54	0.14	-3.88	0.0003
Polyploidy	0.60	0.19	3.18	0.0024
Mollusc : N	1.80	12.52	0.14	0.8861
Mollusc : P	-3.43	13.87	-0.25	0.8058
N : P	-24.75	14.52	-1.70	0.0936
Mollusc : N : P	22.26	19.84	1.12	0.2665

Table S3.13 iii) Insects + molluscs

a) Genome size, var = N

	B	Std.Error	t-value	p-value
Intercept	6.20	0.31	19.92	< 0.0001
ins&mol	1.10	0.42	2.60	0.0116
N	-1.79	0.68	-2.63	0.0108
P	-0.35	0.51	-0.68	0.4998
ins&mol : N	1.03	0.94	1.09	0.2785
ins&mol : P	-1.18	0.71	-1.67	0.1003
N : P	0.61	1.20	0.51	0.6147
ins&mol : N : P	-0.34	1.64	-0.20	0.8385

	B	Std.Error	t-value	p-value
Intercept	-0.17	0.10	-1.71	0.0931
ins&mol	0.01	0.05	0.28	0.7823
N	0.20	0.05	4.20	0.0001
P	0.10	0.05	1.94	0.0571
Ploidy	0.00	0.00	2.15	0.0361
C-value	0.03	0.02	1.97	0.0532
ins&mol : N	-0.09	0.06	-1.50	0.1393
ins&mol : P	-0.05	0.08	-0.67	0.5086
N : P	-0.01	0.08	-0.14	0.8877
ins&mol : N : P	-0.03	0.11	-0.30	0.7675

c) Polyploidy, var = N * ins & mol

	B	Std.Error	t-value	p-value
Intercept	47.61	2.98	15.96	< 0.0001
ins&mol	-1.77	2.06	-0.86	0.3941
N	-1.36	4.54	-0.30	0.7653
P	-3.63	2.77	-1.31	0.1960
C-value	6.92	0.40	17.51	< 0.0001
ins&mol : N	-1.96	4.80	-0.41	0.6847
ins&mol : P	7.19	3.37	2.13	0.0371
N : P	8.75	7.93	1.10	0.2742
ins&mol : N : P	-12.69	8.44	-1.50	0.1378

Table S3.13 iii) continued

d) Herbivore resistant, var = ins & mol

	B	Std.Error	t-value	p-value
Intercept	131.07	10.46	12.52	< 0.0001
ins&mol	5.26	4.67	1.13	0.2649
N	-11.50	6.35	-1.81	0.0757
P	-15.59	6.90	-2.26	0.0276
C-value	11.35	1.65	6.86	< 0.0001
Competition	-66.07	12.06	-5.48	< 0.0001
Ploidy	-0.97	0.19	-5.17	< 0.0001
ins&mol : N	16.19	6.57	2.47	0.0167
ins&mol : P	18.80	7.78	2.42	0.0189
N : P	-0.69	9.93	-0.07	0.9451
ins&mol : N : P	-8.64	11.25	-0.77	0.4454

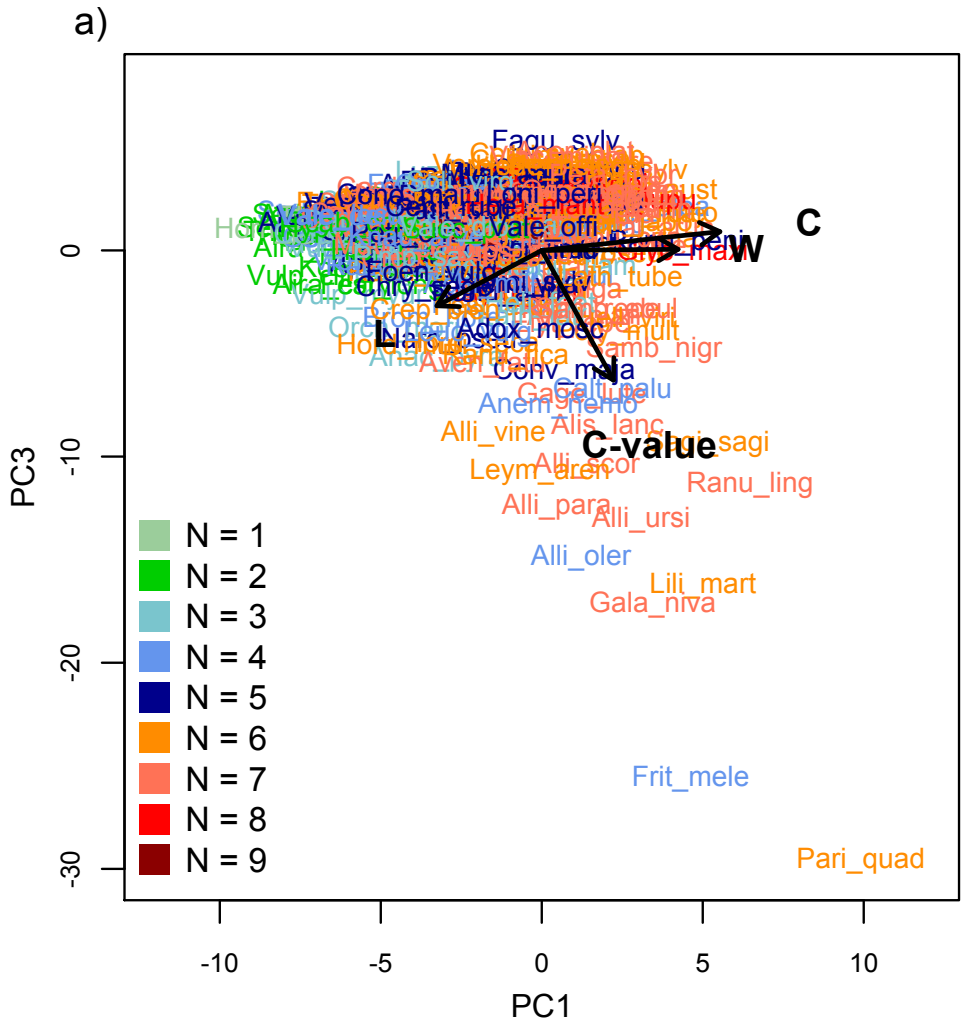
e) Biomass, var= N

	B	Std.Error	t-value	p-value
Intercept	38.57	7.41	5.20	< 0.0001
ins&mol	-11.63	6.29	-1.85	0.0695
N	39.73	9.24	4.30	0.0001
P	6.81	8.92	0.76	0.4479
Competition	36.41	14.17	2.57	0.0128
ins&mol : N	-11.74	10.58	-1.11	0.2716
ins&mol : P	-13.83	10.39	-1.33	0.1881
N : P	-13.49	15.88	-0.85	0.3990
ins&mol : N : P	27.36	18.37	1.49	0.1418

Appendix 3 Supporting information for Chapter 4

Supporting Figures

Supporting Tables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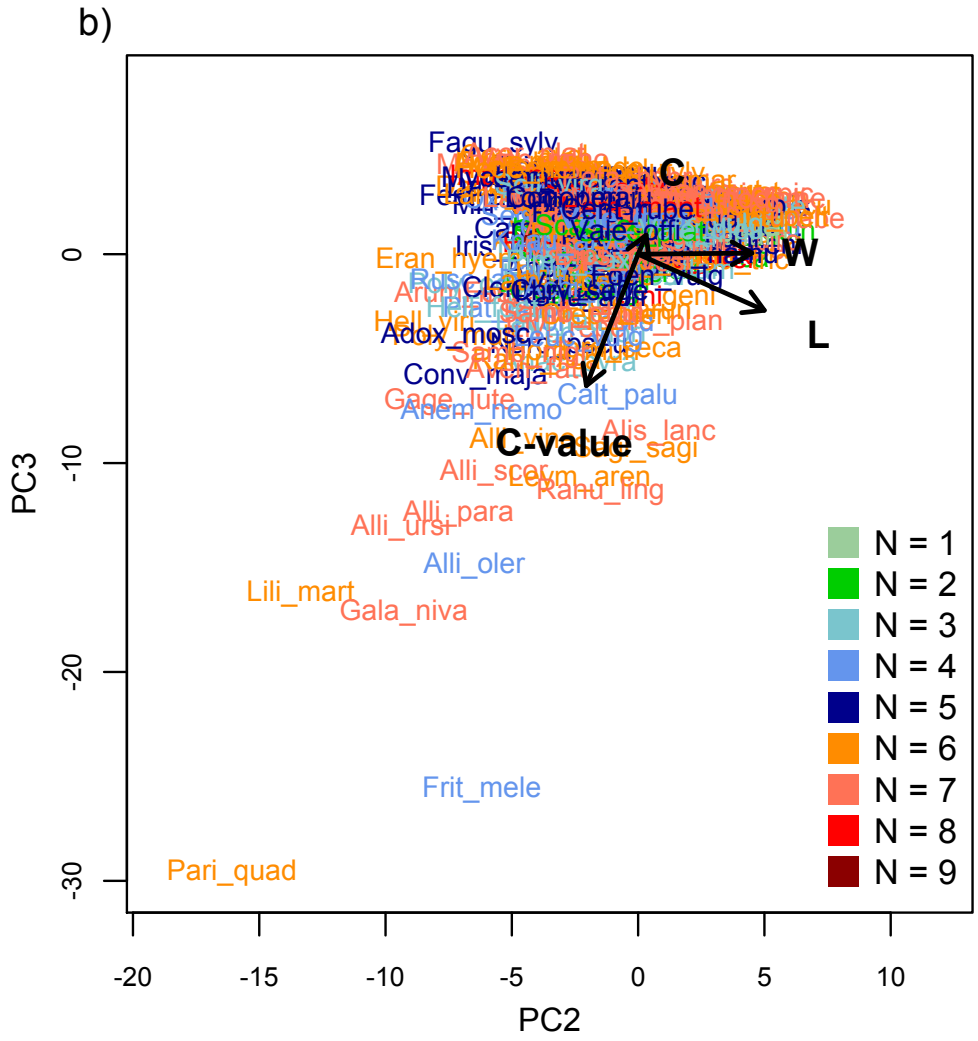

Figure S4. 1 Phylogenetic principal component analysis (PPCA) biplot showing how GS, a species' competitiveness (Grime's C- strategy), and indicator values for water (W) and light (L) map onto a species N attribute on: **a)** principal components (PC) 1 and 3, and **b)** PC2 and PC3. Ellenberg's N value for each species is plotted per the colour scheme shown in the legend.

Table S4.1. List of species and their genome sizes (1C-value), indicator values for light (L), water (W), and nitrogen (N), C-S-R type (Grime, 1977), C-strategy, APG family, chromosome counts, and ploidy level. n= 462. C-values and chromosome counts were obtained from the Plant DNA C-values Database (Bennett & Leitch, 2012).

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Acer_campestre	0.69	5	5	6	SC	0.5	Sapindaceae	26	2
Acer_platanoides	0.71	4	5	7	C/SC	0.75	Sapindaceae	26	2
Acer_pseudoplatanus	1.35	4	5	6	C/SC	0.75	Sapindaceae	52	4
Achillea_millefolium	7.65	7	5	4	CSR	0.3333	Asteraceae	54	6
Achillea_ptarmica	2.9	7	7	3	SC/CSR	0.4167	Asteraceae	18	2
Acorus_calamus	0.65	8	10	7	C/CR	0.75	Acoraceae	NA	NA
Adoxa_moschatellina	14.3	4	5	5	SR	0	Adoxaceae	36	4
Agrimonia_eupatoria	3.98	7	4	4	CSR	0.3333	Rosaceae	28	NA
Agrostis_canina	3.5	7	7	3	SR/CSR	0.1667	Poaceae	NA	NA
Agrostis_capillaris	3.53	6	5	4	CSR	0.3333	Poaceae	28	4
Agrostis_gigantea	2.68	7	6	7	CSR	0.3333	Poaceae	NA	NA
Agrostis_stolonifera	3.5	7	6	6	CR	0.5	Poaceae	NA	NA
Agrostis_vinealis	3.45	7	6	2	CSR	0.3333	Poaceae	NA	NA
Aira_caryophyllea	6.03	8	2	2	SR	0	Poaceae	28	4
Aira_praecox	2.93	8	2	2	SR	0	Poaceae	14	2
Ajuga_reptans	1.2	5	7	5	R/CSR	0.1667	Lamiaceae	NA	NA
Alisma_lanceolatum	18.3	8	10	7	R/CR	0.25	Alismataceae	26	NA
Alisma_plantago-aquatica	10.3	7	10	7	R/CR	0.25	Alismataceae	14	2
Alliaria_petiolata	1.95	5	6	8	CR	0.5	Brassicaceae	36	4
Allium_oleraceum	30.19	7	5	4	S/CSR	0.1667	Amaryllidaceae	40	5
Allium_paradoxum	26.75	6	5	7	SR	0	Amaryllidaceae	16	2
Allium_scorodoprasum	23.63	6	6	7	S/CSR	0.1667	Amaryllidaceae	24	3
Allium_ursinum	30.17	4	6	7	SR	0	Amaryllidaceae	14	2
Allium_vineale	19.53	7	5	6	SR	0	Amaryllidaceae	NA	NA
Alnus_glutinosa	0.55	5	8	6	SC	0.5	Betulaceae	28	2
Alopecurus_geniculatus	7.48	8	7	6	CR	0.5	Poaceae	28	4
Alopecurus_myosuroides	4.33	6	5	6	R	0	Poaceae	NA	NA
Alopecurus_pratensis	6.8	7	5	7	C/CSR	0.6667	Poaceae	NA	NA
Ammophila_arenaria	3.88	9	4	3	SC	0.5	Poaceae	28	4
Anacamptis_pyramidalis	12.32	8	4	3	SR/CSR	0.1667	Orchidaceae	NA	NA
Anemone_nemorosa	19.48	5	6	4	SR	0	Ranunculaceae	30	4

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom. no.	Ploidy level
Anthoxanthum_odoratum	5.9	7	6	3	SR/CSR	0.1667	Poaceae	20	4
Anthriscus_sylvestris	2.25	6	5	7	C/CR	0.75	Apiaceae	16	2
Anthyllis_vulneraria	0.48	8	4	2	SR/CSR	0.1667	Fabaceae	12	2
Antirrhinum_majus	0.65	8	3	5	CSR	0.3333	Plantaginaceae	16	2
Apera_spica-venti	5.4	7	4	5	R/SR	0	Poaceae	14	2
Aphanes_arvensis	0.58	8	4	4	R/SR	0	Rosaceae	48	6
Apium_nodiflorum	1.08	7	10	7	C/CR	0.75	Apiaceae	22	2
Aquilegia_vulgaris	0.51	6	4	5	CSR	0.3333	Ranunculaceae	NA	NA
Arabidopsis_thaliana	0.16	8	3	2	SR	0	Brassicaceae	10	2
Arabis_hirsuta	0.69	7	5	3	S/CSR	0.1667	Brassicaceae	32	4
Arctostaphylos_uva-ursi	1.25	7	5	2	S/SC	0.25	Ericaceae	NA	NA
Arenaria_serpyllifolia	0.8	8	3	5	SR	0	Caryophyllaceae	40	4
Arrhenatherum_elatius	7.98	7	5	7	C/CSR	0.6667	Poaceae	28	4
Artemisia_absinthium	3.65	7	4	9	C/CSR	0.6667	Asteraceae	18	2
Artemisia_vulgaris	3.25	7	4	7	C/CR	0.75	Asteraceae	16	2
Arum_maculatum	10.93	4	5	7	SR	0	Araceae	56	8
Aster_lanceolatus	2.71	7	5	6	C/CSR	0.6667	Asteraceae	NA	8
Astragalus_glycyphyllos	0.75	6	4	3	SC/CSR	0.4167	Fabaceae	16	2
Atriplex_patula	2.15	7	5	7	R/CR	0.25	Amaranthaceae	36	4
Atriplex_prostrata	0.76	8	7	7	R/CR	0.25	Amaranthaceae	18	2
Avena_fatua	14.15	7	4	7	R/CR	0.25	Poaceae	42	6
Bellis_perennis	1.15	8	5	4	R/CSR	0.1667	Asteraceae	18	2
Betula_pubescens	0.9	7	7	4	C/SC	0.75	Betulaceae	56	4
Blackstonia_perfoliata	1.45	8	5	2	SR	0	Gentianaceae	44	4
Brachypodium_pinnatum	0.76	7	3	3	SC	0.5	Poaceae	28	4
Brachypodium_sylvaticum	0.43	6	5	5	SC/CSR	0.4167	Poaceae	18	2
Brassica_nigra	0.78	8	5	6	CR	0.5	Brassicaceae	16	2
Brassica_rapa	0.8	7	5	6	CR	0.5	Brassicaceae	20	2
Briza_media	5.2	8	5	3	S/CSR	0.1667	Poaceae	14	2
Bromus_hordeaceus	9.18	8	4	4	R/CR	0.25	Poaceae	28	4
Bryonia_dioica	1.65	7	5	7	C/CSR	0.6667	Cucurbitaceae	20	2
Butomus_umbellatus	4.9	7	11	7	CR	0.5	Butomaceae	39	3
Callitriche_hamulata	4.35	7	11	5	R/SR	0	Plantaginaceae	38	NA
Callitriche_obtusangula	1.83	7	11	6	R/CR	0.25	Plantaginaceae	10	2
Callitriche_platycarpa	2.78	6	10	7	CR	0.5	Plantaginaceae	20	4

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom. no.	Ploidy level
Callitriche_stagnalis	1.23	7	10	6	R/CR	0.25	Plantaginaceae	10	2
Callitriche_truncata	1.3	7	12	7	SR	0	Plantaginaceae	6	2
Caltha_palustris	16.5	7	9	4	CSR	0.3333	Ranunculaceae	56	7
Calystegia_sepium	0.8	7	8	7	C/CR	0.75	Convolvulaceae	22	2
Campanula_glomerata	1.81	8	4	3	S	0	Campanulaceae	NA	NA
Campanula_rapunculoides	3.98	6	4	5	CR/CSR	0.4167	Campanulaceae	NA	NA
Campanula_rotundifolia	2.65	7	4	2	S/CSR	0.1667	Campanulaceae	68	4
Capsella_bursa-pastoris	0.4	7	5	7	R	0	Brassicaceae	32	4
Cardamine_amara	0.24	6	9	6	CR	0.5	Brassicaceae	16	2
Cardamine_flexuosa	0.88	5	7	6	R/SR	0	Brassicaceae	32	4
Cardamine_hirsuta	0.23	8	5	6	SR	0	Brassicaceae	NA	NA
Cardamine_impatiens	0.21	6	5	7	R	0	Brassicaceae	16	NA
Cardamine_pratensis	1.68	7	8	4	R/CSR	0.1667	Brassicaceae	NA	NA
Carex_acutiformis	0.41	7	9	6	C/SC	0.75	Cyperaceae	NA	NA
Carex_caryophyllea	0.78	7	4	2	S/CSR	0.1667	Cyperaceae	66	2
Carex_flacca	0.3	7	5	2	S/SC	0.25	Cyperaceae	76	NA
Carex_panicea	1	8	8	2	S/CSR	0.1667	Cyperaceae	32	NA
Carex_pulicaris	0.4	8	7	2	S	0	Cyperaceae	60	NA
Carpinus_betulus	1.03	4	5	6	SC	0.5	Betulaceae	NA	NA
Castanea_sativa	0.98	5	5	5	SC	0.5	Fagaceae	NA	NA
Catabrosa_aquatica	3.25	6	9	7	CR	0.5	Poaceae	22	NA
Centaurea_nigra	1.8	7	5	5	CSR	0.3333	Asteraceae	NA	NA
Centaurea_scabiosa	1.68	8	3	3	SC/CSR	0.4167	Asteraceae	20	2
Centaurium_erythraea	1.23	8	5	3	SR	0	Gentianaceae	NA	NA
Centranthus_ruber	0.58	8	4	5	C/CSR	0.6667	Caprifoliaceae	32	4
Cerastium_arvense	1.3	8	4	3	SR/CSR	0.1667	Caryophyllaceae	72	4
Cerastium_fontanum	2.93	7	5	4	R/CSR	0.1667	Caryophyllaceae	144	16
Ceratophyllum_demersum	0.69	7	12	7	CR	0.5	Ceratophyllaceae	NA	6
Chamerion_angustifolium	0.4	6	5	5	C	1	Onagraceae	36	2
Chelidonium_majus	1.2	6	5	7	CR/CSR	0.4167	Papaveraceae	12	2
Chenopodium_album	2.33	7	5	7	CR	0.5	Amaranthaceae	54	6
Chenopodium_ficifolium	0.66	7	6	7	R/CR	0.25	Amaranthaceae	18	2
Chenopodium_murale	0.62	8	6	7	R	0	Amaranthaceae	18	2
Chrysanthemum_segetum	7.35	7	5	5	R	0	Asteraceae	18	2
Cirsium_acaule	1.31	9	4	3	SC/CSR	0.4167	Asteraceae	34	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Cirsium_arvense	1.42	8	6	6	C	1	Asteraceae	34	2
Cirsium_eriophorum	1.8	8	4	5	R/CSR	0.1667	Asteraceae	34	2
Cirsium_heterophyllum	1.07	7	6	5	SC/CSR	0.4167	Asteraceae	34	2
Cirsium_palustre	1.29	7	8	4	CR/CSR	0.4167	Asteraceae	34	2
Cirsium_vulgare	2.77	7	5	6	CR	0.5	Asteraceae	68	4
Clematis_vitalba	9.05	6	4	5	SC	0.5	Ranunculaceae	16	2
Cochlearia_danica	0.7	9	6	5	SR	0	Brassicaceae	NA	NA
Cochlearia_pyrenaica	0.4	8	7	3	SR/CSR	0.1667	Brassicaceae	12	2
Conopodium_majus	0.83	6	5	5	SR	0	Apiaceae	NA	NA
Convallaria_majalis	16.99	5	5	5	S/SC	0.25	Asparagaceae	NA	NA
Convolvulus_arvensis	1.78	7	4	6	CR	0.5	Convolvulaceae	48	NA
Conyza_canadensis	0.45	7	4	6	R/CR	0.25	Asteraceae	NA	2
Cornus_sanguinea	1.16	7	5	6	SC	0.5	Cornaceae	22	2
Corylus_avellana	0.48	4	5	6	SC	0.5	Betulaceae	22	2
Cotoneaster_horizontalis	1.39	8	3	4	SC	0.5	Rosaceae	NA	4
Crataegus_monogyna	0.76	6	5	6	SC	0.5	Rosaceae	34	2
Crepis_biennis	8.45	8	5	6	R/CSR	0.1667	Asteraceae	40	8
Crepis_capillaris	2.1	7	4	4	R/SR	0	Asteraceae	6	2
Crepis_paludosa	4.16	6	7	4	CSR	0.3333	Asteraceae	12	2
Crepis_vesicaria	4.18	8	5	7	R/CR	0.25	Asteraceae	8	2
Cymbalaria_muralis	0.49	7	5	6	R/CSR	0.1667	Plantaginaceae	NA	NA
Cynosurus_cristatus	3.05	7	5	4	R/CSR	0.1667	Poaceae	14	2
Cytisus_scoparius	0.85	8	5	4	SC	0.5	Fabaceae	48	4
Dactylis_glomerata	4.4	7	5	6	C/CSR	0.6667	Poaceae	28	4
Dactylorhiza_fuchsii	2.89	7	8	3	SR	0	Orchidaceae	40	2
Dactylorhiza_incarnata	3.55	8	9	2	SR	0	Orchidaceae	40	2
Dactylorhiza_maculata	5.66	7	7	2	SR	0	Orchidaceae	80	4
Danthonia_decumbens	2.95	7	6	2	S/CSR	0.1667	Poaceae	36	4
Daphne_laureola	2.99	4	5	5	SC	0.5	Thymelaeaceae	18	2
Daphne_mezereum	3.03	4	5	6	SC	0.5	Thymelaeaceae	18	2
Daucus_carota	1	8	4	3	SR/CSR	0.1667	Apiaceae	18	2
Deschampsia_flexuosa	5.48	6	5	3	S/SC	0.25	Poaceae	28	4
Digitalis_purpurea	1.23	6	6	5	SR/CSR	0.1667	Plantaginaceae	56	2
Dipsacus_fullonum	3.28	8	7	7	CR	0.5	Caprifoliaceae	18	2
Dipsacus_pilosus	5.29	7	6	7	CR	0.5	Caprifoliaceae	18	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Drosera_intermedia	0.95	8	9	1	S/SR	0	Droseraceae	20	2
Drosera_rotundifolia	0.88	8	9	1	SR	0	Droseraceae	20	2
Eleocharis_acicularis	1.24	7	10	5	R/SR	0	Cyperaceae	20	NA
Eleocharis_palustris	2.1	8	10	4	C/CSR	0.6667	Cyperaceae	16	NA
Eleocharis_quinqueflora	0.56	9	9	2	S/SC	0.25	Cyperaceae	136	NA
Elodea_canadensis	4.18	7	12	6	CR	0.5	Hydrocharitaceae	48	NA
Elymus_caninus	8.55	7	6	8	SC/CSR	0.4167	Poaceae	28	4
Elytrigia_repens	11.64	7	5	7	C/CR	0.75	Poaceae	42	6
Empetrum_nigrum	0.65	7	6	1	SC	0.5	Ericaceae	26	2
Epilobium_ciliatum	0.53	7	6	6	R/CSR	0.1667	Onagraceae	36	2
Epilobium_hirsutum	0.3	7	8	7	C	1	Onagraceae	36	2
Epilobium_obscurum	0.25	6	8	5	CR/CSR	0.4167	Onagraceae	36	2
Epilobium_palustre	0.15	7	8	3	S/CSR	0.1667	Onagraceae	36	2
Epilobium_tetragonum	0.58	6	7	5	R/CSR	0.1667	Onagraceae	36	2
Eranthis_hyemalis	9.3	3	5	6	SR	0	Ranunculaceae	16	2
Eriophorum_angustifolium	0.65	8	9	1	S/SC	0.25	Cyperaceae	58	2
Eriophorum_vaginatum	0.38	8	8	1	S/SC	0.25	Cyperaceae	NA	NA
Erysimum_cheiranthoides	0.83	7	5	7	R	0	Brassicaceae	NA	NA
Euonymus_europaeus	0.94	5	5	5	SC	0.5	Celastraceae	64	4
Eupatorium_cannabinum	2.58	7	8	7	C	1	Asteraceae	20	2
Euphorbia_cyparissias	1.11	8	3	3	CSR	0.3333	Euphorbiaceae	40	NA
Euphorbia_peplus	0.35	7	4	6	R	0	Euphorbiaceae	22	2
Fagus_sylvatica	0.56	3	5	5	SC	0.5	Fagaceae	24	2
Fallopia_convolvulus	0.73	7	4	5	R/CR	0.25	Polygonaceae	40	4
Fallopia_japonica	4.82	6	7	6	C	1	Polygonaceae	88	8
Fallopia_sachalinensis	2.16	6	5	7	C	1	Polygonaceae	44	4
Festuca_altissima	4.47	3	5	5	S/SC	0.25	Poaceae	14	2
Festuca_arundinacea	8.49	8	6	6	SC/CSR	0.4167	Poaceae	42	6
Festuca_gigantea	10.38	5	6	7	CSR	0.3333	Poaceae	42	6
Festuca_longifolia	6.35	8	3	2	S/CSR	0.1667	Poaceae	42	6
Festuca_ovina	2.41	7	5	2	S	0	Poaceae	14	2
Festuca_pratensis	2.23	7	6	6	CSR	0.3333	Poaceae	14	2
Festuca_rubra	4.73	8	5	5	CSR	0.3333	Poaceae	42	6
Foeniculum_vulgare	4.55	9	5	5	SC/CSR	0.4167	Apiaceae	22	2
Fragaria_vesca	0.25	6	5	4	S/CSR	0.1667	Rosaceae	14	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Fraxinus_excelsior	0.98	5	6	6	C/SC	0.75	Oleaceae	46	2
Fritillaria_meleagris	47.3	8	8	4	SR	0	Liliaceae	24	2
Fumaria_muralis	0.55	7	5	6	R/CR	0.25	Papaveraceae	28	4
Gagea_lutea	19.75	4	6	7	SR	0	Liliaceae	72	6
Galanthus_nivalis	36.1	5	6	7	SR	0	Amaryllidaceae	24	2
Galega_officinalis	2.21	8	5	8	C/CSR	0.6667	Fabaceae	NA	2
Galinsoga_parviflora	1.25	7	4	7	R	0	Asteraceae	16	2
Galium_aparine	1.03	6	6	8	CR	0.5	Rubiaceae	NA	NA
Galium_mollugo	1.88	7	4	4	C/CSR	0.6667	Rubiaceae	44	NA
Galium_palustre	1.3	7	9	4	CR/CSR	0.4167	Rubiaceae	NA	NA
Galium_saxatile	1.45	6	6	3	S/CSR	0.1667	Rubiaceae	44	4
Galium_sterneri	1	9	4	1	S/CSR	0.1667	Rubiaceae	44	4
Galium_verum	1.89	7	4	2	SC/CSR	0.4167	Rubiaceae	44	4
Genista_tinctoria	1.67	8	6	2	SC	0.5	Fabaceae	96	4
Geranium_pyrenaicum	1.44	8	4	6	CSR	0.3333	Geraniaceae	NA	2
Glyceria_fluitans	1.73	7	10	6	CR	0.5	Poaceae	40	4
Glyceria_maxima	6.13	7	10	8	C	1	Poaceae	60	6
Gymnadenia_conopsea	5.51	7	6	3	SR	0	Orchidaceae	NA	NA
Hedera_helix	1.48	4	5	6	SC	0.5	Araliaceae	48	4
Helianthemum_nummularium	2.23	7	4	2	S	0	Cistaceae	20	4
Helictotrichon_pubescens	6.28	7	4	3	S/CSR	0.1667	Poaceae	14	2
Helleborus_foetidus	11.65	5	4	3	SC/CSR	0.4167	Ranunculaceae	32	2
Helleborus_viridis	15.2	3	5	6	SC/CSR	0.4167	Ranunculaceae	32	2
Heracleum_mantegazzianum	1.78	7	6	8	C/CR	0.75	Apiaceae	NA	2
Heracleum_sphondylium	2.19	7	5	7	C/CSR	0.6667	Apiaceae	22	NA
Hesperis_matronalis	3.8	7	7	7	CR/CSR	0.4167	Brassicaceae	NA	4
Hieracium_pilosella	3.45	8	4	2	S/CSR	0.1667	Asteraceae	36	4
Hippocrepis_comosa	1.9	8	3	2	S	0	Fabaceae	28	NA
Holcus_lanatus	1.7	7	6	5	CSR	0.3333	Poaceae	14	2
Holcus_mollis	4.1	6	6	3	C/CSR	0.6667	Poaceae	35	5
Hordeum_murinum	11.1	8	4	6	R	0	Poaceae	28	4
Hordeum_secalinum	11.2	8	6	6	R/CSR	0.1667	Poaceae	28	4
Hornungia_petraea	0.17	9	2	1	SR	0	Brassicaceae	12	2
Humulus_lupulus	2.9	6	7	8	C	1	Cannabaceae	NA	NA
Hydrocotyle_vulgaris	0.98	8	8	3	R/CSR	0.1667	Araliaceae	NA	NA

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Hypericum_hirsutum	0.15	6	5	5	SC/CSR	0.4167	Hypericaceae	NA	NA
Hypericum_perforatum	0.78	7	4	5	CR/CSR	0.4167	Hypericaceae	32	4
Hypochaeris_glabra	1.3	8	4	2	SR	0	Asteraceae	NA	NA
Hypochaeris_radicata	1.34	8	4	3	CSR	0.3333	Asteraceae	8	2
Ilex_aquifolium	1.15	5	5	5	SC	0.5	Aquifoliaceae	40	4
Impatiens_glandulifera	1.15	6	8	7	CR	0.5	Balsaminaceae	18	2
Impatiens_parviflora	2.13	4	5	8	CR	0.5	Balsaminaceae	NA	2
Inula_helenium	2.26	6	6	5	C/CSR	0.6667	Asteraceae	NA	2
Iris_foetidissima	7.23	5	4	5	C/SC	0.75	Iridaceae	NA	NA
Iris_germanica	5.87	8	4	4	C/CSR	0.6667	Iridaceae	24	2
Iris_pseudacorus	5.67	7	9	6	C/CSR	0.6667	Iridaceae	24	2
Juncus_articulatus	1.83	8	9	3	CR/CSR	0.4167	Juncaceae	80	8
Juncus_bufonius	1.3	7	7	5	R/SR	0	Juncaceae	NA	NA
Juncus_effusus	0.3	7	7	4	C/SC	0.75	Juncaceae	46	2
Juncus_squarrosus	0.53	7	7	2	S/CSR	0.1667	Juncaceae	40	4
Juncus_tenuis	0.46	7	7	4	R/CSR	0.1667	Juncaceae	NA	8
Knautia_arvensis	3.69	7	3	4	CSR	0.3333	Caprifoliaceae	20	2
Koeleria_macrantha	4.66	8	4	2	S	0	Poaceae	28	4
Lactuca_virosa	3.76	8	4	7	CR	0.5	Asteraceae	18	2
Lagarosiphon_major	3.25	6	12	6	CR	0.5	Hydrocharitaceae	22	NA
Lamiastrum_galeobdolon	3.25	4	5	6	S/SC	0.25	Lamiaceae	18	2
Lamium_album	1.1	7	5	8	CR	0.5	Lamiaceae	18	2
Lamium_purpureum	1.1	6	5	7	R	0	Lamiaceae	18	2
Lapsana_communis	1.18	6	4	7	R/CR	0.25	Asteraceae	12	2
Lathyrus_latifolius	10.88	7	4	3	C/CSR	0.6667	Fabaceae	14	2
Lathyrus_pratensis	4.54	7	6	5	CSR	0.3333	Fabaceae	14	NA
Lathyrus_tuberosus	9.3	6	5	6	C	1	Fabaceae	14	2
Lemna_minor	0.6	7	11	6	CR	0.5	Araceae	40	4
Lemna_trisulca	0.46	7	12	5	SR	0	Araceae	NA	NA
Leontodon_autumnalis	1.16	8	6	4	R/CSR	0.1667	Asteraceae	12	2
Leontodon_hispidus	2.5	8	4	3	CSR	0.3333	Asteraceae	14	2
Lepidium_latifolium	1.04	8	5	8	C/CSR	0.6667	Brassicaceae	40	NA
Leucanthemum_vulgare	10.65	8	4	4	CR/CSR	0.4167	Asteraceae	36	4
Leymus_arenarius	21.25	9	5	6	SC	0.5	Poaceae	NA	NA
Ligustrum_vulgare	1.57	6	5	5	SC	0.5	Oleaceae	46	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Lilium_martagon	37.19	3	4	6	S/SC	0.25	Liliaceae	24	2
Linaria_vulgaris	0.87	7	4	6	CR/CSR	0.4167	Plantaginaceae	NA	NA
Lolium_multiflorum	2.72	7	5	7	R/CR	0.25	Poaceae	14	2
Lolium_perenne	2.76	8	5	6	CR/CSR	0.4167	Poaceae	14	2
Lonicera_periclymenum	2.75	5	6	5	SC	0.5	Caprifoliaceae	NA	NA
Lotus_corniculatus	1.05	7	4	2	S/CSR	0.1667	Fabaceae	24	4
Lotus_pedunculatus	0.55	7	8	4	C/CSR	0.6667	Fabaceae	12	2
Lupinus_arboreus	0.9	9	4	3	SC	0.5	Fabaceae	NA	NA
Luzula_campestris	0.49	7	4	2	S/CSR	0.1667	Juncaceae	12	2
Luzula_multiflora	1.37	7	6	3	S/CSR	0.1667	Juncaceae	36	6
Luzula_pilosa	0.28	5	5	3	S/CSR	0.1667	Juncaceae	66	NA
Luzula_sylvatica	0.78	5	5	4	SC	0.5	Juncaceae	12	NA
Lysimachia_punctata	2.21	6	6	5	C/CSR	0.6667	Primulaceae	NA	2
Malus_sylvestris	0.74	7	5	6	SC	0.5	Rosaceae	34	2
Malva_sylvestris	1.48	8	4	7	CR	0.5	Malvaceae	42	6
Matricaria_chamomilla	3.88	7	5	7	R	0	Asteraceae	18	2
Matricaria_discoidea	2.45	7	5	7	R	0	Asteraceae	18	2
Medicago_lupulina	0.88	7	4	4	R/CSR	0.1667	Fabaceae	16	2
Medicago_sativa	1.75	7	4	5	C/CSR	0.6667	Fabaceae	32	4
Melilotus_albus	1.33	9	3	4	CR	0.5	Fabaceae	16	2
Melilotus_altissimus	1.23	8	6	7	CR/CSR	0.4167	Fabaceae	16	2
Melilotus_officinalis	1.13	8	5	5	CR/CSR	0.4167	Fabaceae	16	2
Mentha_aquatica	1.5	7	8	5	C/CR	0.75	Lamiaceae	96	NA
Mercurialis_annua	1.3	7	5	7	R/CR	0.25	Euphorbiaceae	32	4
Mercurialis_perennis	2.35	3	6	7	SC	0.5	Euphorbiaceae	64	8
Milium_effusum	3.95	4	5	5	S/CSR	0.1667	Poaceae	28	4
Mimulus_guttatus	0.37	7	9	6	CR	0.5	Phrymaceae	NA	4
Minuartia_verna	0.58	8	4	1	S	0	Caryophyllaceae	NA	NA
Molinia_caerulea	2.45	7	8	2	SC	0.5	Poaceae	36	4
Mycelis_muralis	2	4	5	5	CSR	0.3333	Asteraceae	18	2
Myosotis_scorpioides	1.4	7	9	6	CR	0.5	Boraginaceae	64	NA
Myriophyllum_alterniflorum	0.55	7	12	3	SR	0	Haloragaceae	28	4
Myriophyllum_spicatum	0.25	7	12	7	CR	0.5	Haloragaceae	14	2
Myrrhis_odorata	0.85	7	6	7	C/CSR	0.6667	Apiaceae	22	2
Narcissus_pseudonarcissus	11.75	7	5	5	SR	0	Amaryllidaceae	14	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Nardus_stricta	2.1	7	7	2	S/SC	0.25	Poaceae	26	2
Narthecium_ossifragum	0.41	8	9	1	S/CSR	0.1667	Nartheciaceae	26	2
Nuphar_lutea	2.53	7	11	6	C/CR	0.75	Nymphaeaceae	NA	NA
Nymphaea_alba	1.99	7	11	4	C/CR	0.75	Nymphaeaceae	NA	NA
Nymphoides_peltata	0.73	8	11	6	CSR	0.3333	Menyanthaceae	54	NA
Odontites_vernus	0.57	7	5	5	R/CR	0.25	Orobanchaceae	NA	2
Oenanthe_crocata	0.65	7	9	7	C/CR	0.75	Apiaceae	22	2
Oenanthe_fistulosa	0.63	7	9	6	SR/CSR	0.1667	Apiaceae	22	2
Oenothera_biennis	1.22	9	4	4	R/CSR	0.1667	Onagraceae	14	2
Onobrychis_viciifolia	1.25	7	4	3	CSR	0.3333	Fabaceae	28	4
Ononis_repens	1.41	8	4	3	SC/CSR	0.4167	Fabaceae	NA	NA
Onopordum_acanthium	1.33	8	4	7	CR	0.5	Asteraceae	34	2
Orchis_morio	9.64	8	4	3	SR	0	Orchidaceae	NA	NA
Origanum_vulgare	0.68	6	4	4	SC/CSR	0.4167	Lamiaceae	30	3
Oxalis_acetosella	3.2	4	6	4	S/SR	0	Oxalidaceae	22	2
Oxalis_corniculata	1.45	7	4	5	R	0	Oxalidaceae	NA	NA
Papaver_dubium	4.5	7	5	5	R	0	Papaveraceae	28	4
Papaver_rhoeas	2.63	7	5	6	R	0	Papaveraceae	14	2
Papaver_somniferum	3.8	7	4	8	R/CR	0.25	Papaveraceae	22	2
Paris_quadrifolia	60.1	3	6	6	SR/CSR	0.1667	Melanthiaceae	NA	NA
Parnassia_palustris	1.18	8	8	3	SR/CSR	0.1667	Celastraceae	NA	NA
Pastinaca_sativa	1.73	7	4	5	CR	0.5	Apiaceae	22	2
Persicaria_lapathifolia	0.7	7	6	7	CR	0.5	Polygonaceae	22	2
Persicaria_maculosa	0.43	7	6	7	R/CR	0.25	Polygonaceae	44	4
Petasites_hybridus	0.88	6	7	7	C	1	Asteraceae	60	2
Petroselinum_crispum	2.25	8	4	5	CSR	0.3333	Apiaceae	NA	NA
Phalaris_arundinacea	4.13	7	9	7	C	1	Poaceae	28	4
Phleum_bertolonii	1.7	8	4	4	SR/CSR	0.1667	Poaceae	14	2
Phleum_pratense	4.15	8	5	6	CSR	0.3333	Poaceae	42	6
Phragmites_australis	1	7	10	6	C	1	Poaceae	NA	2
Picris_echioides	1.2	7	5	6	CR	0.5	Asteraceae	10	2
Picris_hieracioides	1.58	8	4	3	R/CSR	0.1667	Asteraceae	10	2
Pimpinella_major	3.23	7	5	6	CSR	0.3333	Apiaceae	18	2
Pimpinella_saxifraga	5.13	7	4	3	SR/CSR	0.1667	Apiaceae	36	4
Plantago_coronopus	0.86	8	6	4	SR/CSR	0.1667	Plantaginaceae	10	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Plantago_lanceolata	1.2	7	5	4	CSR	0.3333	Plantaginaceae	12	2
Plantago_major	0.71	7	5	7	R/CSR	0.1667	Plantaginaceae	12	2
Plantago_media	2.78	8	4	3	S/CSR	0.1667	Plantaginaceae	24	4
Platanthera_chlorantha	11.06	5	5	4	SR	0	Orchidaceae	NA	NA
Poa_annua	2.88	7	5	7	R	0	Poaceae	28	4
Poa_pratensis	4.24	7	5	5	CSR	0.3333	Poaceae	NA	NA
Poa_trivialis	2.83	7	6	6	R/CSR	0.1667	Poaceae	14	2
Polygala_vulgaris	0.45	8	5	3	S/CSR	0.1667	Polygalaceae	NA	NA
Polygonatum_multiflorum	15.35	4	5	6	SC	0.5	Asparagaceae	NA	NA
Polygonatum_odoratum	9.83	5	3	3	SC	0.5	Asparagaceae	18	2
Polygonum_aviculare	0.85	7	5	7	R	0	Polygonaceae	40	4
Populus_tremula	0.45	6	5	6	SC	0.5	Salicaceae	38	2
Quercus_cerris	0.95	6	4	6	SC	0.5	Fagaceae	24	2
Quercus_petraea	0.8	6	6	4	SC	0.5	Fagaceae	24	2
Quercus_robur	0.93	7	5	4	SC	0.5	Fagaceae	24	2
Ranunculus_acris	4.45	7	6	4	CSR	0.3333	Ranunculaceae	14	2
Ranunculus_arvensis	6.13	7	5	6	R	0	Ranunculaceae	32	4
Ranunculus_auricomus	9	6	7	5	SR	0	Ranunculaceae	32	4
Ranunculus_bulbosus	5.63	7	4	4	SR	0	Ranunculaceae	16	2
Ranunculus_circinatus	4.05	7	12	7	CR/CSR	0.4167	Ranunculaceae	32	4
Ranunculus_ficaria	14.18	6	6	6	SR	0	Ranunculaceae	24	3
Ranunculus_flammula	6.35	7	9	3	CR/CSR	0.4167	Ranunculaceae	32	4
Ranunculus_hederaceus	2.1	7	10	5	R	0	Ranunculaceae	16	2
Ranunculus_lingua	25.1	7	10	7	C/CR	0.75	Ranunculaceae	128	16
Ranunculus_penicillatus	4.9	7	12	5	C/CR	0.75	Ranunculaceae	32	4
Ranunculus_repens	11.2	6	7	7	CR	0.5	Ranunculaceae	32	4
Ranunculus_sceleratus	4	8	8	8	R/CR	0.25	Ranunculaceae	32	4
Ranunculus_trichophyllus	4.88	7	12	6	R	0	Ranunculaceae	32	4
Reseda_luteola	0.51	7	4	6	R/CR	0.25	Resedaceae	26	2
Rhinanthus_minor	3.95	7	5	4	R/SR	0	Orobanchaceae	14	2
Ribes_alpinum	1.01	5	5	6	SC	0.5	Grossulariaceae	16	2
Ribes_rubrum	0.97	5	7	6	SC	0.5	Grossulariaceae	16	2
Ribes_uva-crispa	0.94	5	5	6	SC	0.5	Grossulariaceae	16	2
Rorippa_nasturtium-aquaticum	0.73	7	10	7	CR	0.5	Brassicaceae	18	NA
Rosa_arvensis	0.55	6	4	5	SC	0.5	Rosaceae	14	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Rosa_canina	1.43	6	5	6	SC	0.5	Rosaceae	35	5
Rubus_chamaemorus	1.23	9	7	1	SC/CSR	0.4167	Rosaceae	56	8
Rubus_idaeus	0.3	6	5	5	SC	0.5	Rosaceae	NA	NA
Rumex_acetosa	1.65	7	5	4	CSR	0.3333	Polygonaceae	14	2
Rumex_acetosella	1.68	7	5	3	SR/CSR	0.1667	Polygonaceae	42	4
Rumex_crispus	4.4	8	6	6	CR/CSR	0.4167	Polygonaceae	60	6
Rumex_obtusifolius	1.53	7	5	9	C/CSR	0.6667	Polygonaceae	40	4
Ruscus_aculeatus	10.27	4	5	4	S/SC	0.25	Asparagaceae	NA	NA
Sagittaria_sagittifolia	21.25	7	11	6	CR	0.5	Alismataceae	NA	NA
Salix_caprea	0.43	7	7	7	C/SC	0.75	Salicaceae	38	2
Salix_cinerea	0.85	7	8	5	C/SC	0.75	Salicaceae	76	4
Salix_fragilis	0.86	6	8	7	C/SC	0.75	Salicaceae	76	4
Salix_purpurea	0.47	8	9	5	C/SC	0.75	Salicaceae	38	2
Salix_viminalis	0.41	7	8	6	C/SC	0.75	Salicaceae	38	2
Sambucus_ebulus	10.84	7	5	7	C	1	Adoxaceae	NA	NA
Sambucus_nigra	15.25	6	5	7	C	1	Adoxaceae	36	2
Sanguisorba_minor	0.55	7	4	3	S/CSR	0.1667	Rosaceae	28	4
Saponaria_officinalis	2.27	8	5	6	C/CR	0.75	Caryophyllaceae	NA	NA
Saxifraga_granulata	2.38	8	5	4	SR/CSR	0.1667	Saxifragaceae	52	NA
Scabiosa_columbaria	1.07	8	3	2	SR/CSR	0.1667	Caprifoliaceae	16	2
Schoenoplectus_lacustris	0.5	8	11	6	C/SC	0.75	Cyperaceae	38	NA
Schoenoplectus_tabernaemontani	0.4	9	10	7	C/SC	0.75	Cyperaceae	42	NA
Scirpus_sylvaticus	0.45	6	8	6	C	1	Cyperaceae	62	NA
Scrophularia_nodosa	0.69	5	6	6	C/CR	0.75	Scrophulariaceae	NA	NA
Sedum_acre	1.25	8	2	2	S/SR	0	Crassulaceae	NA	NA
Sedum_album	0.15	8	3	2	S	0	Crassulaceae	34	2
Sedum_rupestre	1.04	7	2	4	S/SR	0	Crassulaceae	64	4
Senecio_aquaticus	1.8	7	8	5	R/CR	0.25	Asteraceae	40	4
Senecio_jacobaea	2.25	7	4	4	CR/CSR	0.4167	Asteraceae	40	4
Senecio_squalidus	0.9	8	4	7	R/CSR	0.1667	Asteraceae	20	2
Senecio_viscosus	1.55	8	5	6	R/CR	0.25	Asteraceae	NA	NA
Senecio_vulgaris	1.58	7	5	7	R	0	Asteraceae	40	4
Silene_dioica	2.7	5	6	7	CSR	0.3333	Caryophyllaceae	24	2
Silene_latifolia	2.7	7	4	6	R/CR	0.25	Caryophyllaceae	24	2
Silene_nutans	2.39	8	3	4	S/CSR	0.1667	Caryophyllaceae	24	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Silene_vulgaris	1.13	7	4	5	CSR	0.3333	Caryophyllaceae	24	2
Sinapis_arvensis	0.38	8	5	7	R/CR	0.25	Brassicaceae	18	2
Sisymbrium_altissimum	0.26	8	5	4	R/CR	0.25	Brassicaceae	NA	2
Sisymbrium_officinale	0.24	7	4	7	R/CR	0.25	Brassicaceae	14	2
Sisymbrium_orientale	0.31	7	4	5	R/CR	0.25	Brassicaceae	14	NA
Solanum_dulcamara	0.8	7	8	7	C/CSR	0.6667	Solanaceae	24	2
Solanum_nigrum	3.1	7	5	8	R/CR	0.25	Solanaceae	72	6
Solidago_canadensis	1.58	8	5	6	C	1	Asteraceae	18	2
Solidago_gigantea	1.82	8	5	6	C	1	Asteraceae	NA	4
Solidago_virgaurea	1.13	5	5	3	S/CSR	0.1667	Asteraceae	18	2
Sonchus_asper	1.85	7	5	6	R/CR	0.25	Asteraceae	18	2
Sonchus_oleraceus	1.6	7	5	7	R/CR	0.25	Asteraceae	32	4
Sorbus_aucuparia	0.71	6	6	4	SC	0.5	Rosaceae	34	2
Sparganium_erectum	0.53	7	10	7	C/CR	0.75	Typhaceae	30	2
Spergula_arvensis	1.05	7	4	5	R/SR	0	Caryophyllaceae	18	NA
Spirodela_polyrhiza	0.3	7	11	7	R	0	Araceae	80	4
Stachys_officinalis	4.53	7	5	3	S/CSR	0.1667	Lamiaceae	16	2
Stachys_sylvatica	1.28	6	6	8	C/CR	0.75	Lamiaceae	NA	NA
Stellaria_holostea	1.45	5	5	6	CSR	0.3333	Caryophyllaceae	26	2
Stellaria_media	1.05	7	5	7	R	0	Caryophyllaceae	42	7
Succisa_pratensis	2.78	7	7	2	S/CSR	0.1667	Caprifoliaceae	20	2
Symphytum_tuberosum	2.75	6	6	6	C/CSR	0.6667	Boraginaceae	64	8
Syringa_vulgaris	1.2	6	5	5	SC	0.5	Oleaceae	NA	NA
Tamus_communis	0.86	6	5	6	C/CSR	0.6667	Dioscoreaceae	NA	NA
Tanacetum_vulgare	3.93	7	6	7	C/CSR	0.6667	Asteraceae	18	2
Teucrium_scorodonia	1.18	6	4	3	SC/CSR	0.4167	Lamiaceae	34	2
Thlaspi_arvense	0.52	7	4	6	R	0	Brassicaceae	14	2
Thlaspi_caerulescens	0.34	8	4	1	S/SR	0	Brassicaceae	14	2
Torilis_japonica	2.28	7	5	7	R/CR	0.25	Apiaceae	12	2
Tragopogon_pratensis	2.77	8	4	5	CR/CSR	0.4167	Asteraceae	12	2
Trientalis_europaea	2.56	5	6	3	S/CSR	0.1667	Primulaceae	NA	NA
Trifolium_arvense	0.39	9	3	2	R/SR	0	Fabaceae	14	2
Trifolium_campestre	0.37	8	4	4	R/SR	0	Fabaceae	14	2
Trifolium_dubium	0.73	7	4	5	R/SR	0	Fabaceae	28	4
Trifolium_fragiferum	0.54	8	7	6	CR/CSR	0.4167	Fabaceae	16	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Trifolium_hybridum	0.62	7	5	6	CSR	0.3333	Fabaceae	16	2
Trifolium_medium	3.23	7	4	4	SC/CSR	0.4167	Fabaceae	80	10
Trifolium_pratense	0.43	7	5	5	CSR	0.3333	Fabaceae	14	2
Trifolium_repens	1.12	7	5	6	CR/CSR	0.4167	Fabaceae	32	4
Trifolium_striatum	0.38	8	3	2	R/SR	0	Fabaceae	14	2
Trisetum_flavescens	2.55	7	4	4	CSR	0.3333	Poaceae	NA	NA
Trollius_europaeus	4.42	7	7	4	SC/CSR	0.4167	Ranunculaceae	NA	NA
Tussilago_farfara	2.3	7	6	6	C/CR	0.75	Asteraceae	60	2
Typha_latifolia	0.33	8	10	7	C	1	Typhaceae	30	2
Ulex_europaeus	3.85	7	5	3	SC	0.5	Fabaceae	96	6
Ulex_gallii	2.9	7	6	2	SC	0.5	Fabaceae	80	NA
Ulmus_glabra	1.08	4	5	6	C/SC	0.75	Ulmaceae	28	2
Urtica_dioica	1.58	6	6	8	C	1	Urticaceae	52	4
Urtica_urens	0.33	8	5	8	R/CR	0.25	Urticaceae	NA	NA
Valeriana_dioica	1.51	8	8	3	SR/CSR	0.1667	Caprifoliaceae	NA	NA
Valeriana_officinalis	4.08	6	8	5	CSR	0.3333	Caprifoliaceae	NA	8
Valerianella_dentata	0.25	8	4	4	SR	0	Caprifoliaceae	16	2
Valerianella_locusta	0.22	8	4	4	SR	0	Caprifoliaceae	16	2
Veronica_agrestis	0.73	7	6	7	R/SR	0	Plantaginaceae	28	4
Veronica_anagallis-aquatica	1.08	7	10	7	CR	0.5	Plantaginaceae	36	4
Veronica_arvensis	0.33	8	4	5	SR	0	Plantaginaceae	18	2
Veronica_beccabunga	0.73	7	10	6	CR	0.5	Plantaginaceae	NA	NA
Veronica_chamaedrys	1.49	6	5	5	CSR	0.3333	Plantaginaceae	32	4
Veronica_filiformis	0.36	7	6	7	R/CSR	0.1667	Plantaginaceae	14	2
Veronica_hederifolia	1.41	6	5	6	R/SR	0	Plantaginaceae	54	6
Veronica_montana	0.85	4	6	6	SR/CSR	0.1667	Plantaginaceae	18	2
Veronica_officinalis	0.9	6	5	4	SR/CSR	0.1667	Plantaginaceae	36	4
Veronica_persica	0.78	6	5	7	R	0	Plantaginaceae	28	4
Veronica_polita	0.42	7	4	5	R/SR	0	Plantaginaceae	14	2
Veronica_serpyllifolia	0.44	7	5	5	R/CSR	0.1667	Plantaginaceae	14	2
Viburnum_opulus	4.15	6	7	6	SC	0.5	Adoxaceae	18	2
Vicia_cracca	5.3	7	6	5	C/CSR	0.6667	Fabaceae	28	4
Vicia_hirsuta	3.98	7	5	6	R/CR	0.25	Fabaceae	14	2
Vicia_lathyroides	2.63	8	3	3	SR	0	Fabaceae	12	2
Vicia_sativa	2.25	7	4	4	R/CR	0.25	Fabaceae	12	2

Table S4.1 continued

Taxa	1C (pg)	L	W	N	C-S-R type	C-strat	Family	Chrom no.	Ploidy level
Vicia_sepium	4.68	6	5	6	C/CSR	0.6667	Fabaceae	14	2
Vicia_sylvatica	8.08	7	5	5	C/CSR	0.6667	Fabaceae	14	2
Vicia_tetrasperma	3.6	7	5	6	R/CR	0.25	Fabaceae	14	2
Vinca_major	2.1	5	6	6	C/SC	0.75	Apocynaceae	NA	NA
Vinca_minor	0.76	4	6	7	SC	0.5	Apocynaceae	46	NA
Viola_hirta	1.51	7	4	2	S/CSR	0.1667	Violaceae	40	4
Viola_riviniana	1.35	6	5	4	S/CSR	0.1667	Violaceae	40	4
Viola_tricolor	4.04	8	4	4	R/SR	0	Violaceae	26	2
Vulpia_bromoides	2.93	8	4	3	SR	0	Poaceae	14	2
Vulpia_ciliata	4.14	9	2	2	SR	0	Poaceae	28	4
Vulpia_myuros	6.89	8	3	3	R/SR	0	Poaceae	42	6

Table S4.2: Summary statistics for each trait and Ellenberg indicator value for: **a**) all taxa in dataset; **b**) in diploid taxa; and **c**) in polyploid taxa.

a) All taxa, n=462	Mean ± stdev	Median	Mode	Range
Nitrogen	5.03 ± 1.78	5	6	1 - 9
Light	6.84 ± 1.18	7	7	3 - 9
Water	5.78 ± 2.10	5	5	2 - 12
Competition	0.34 ± 0.27	0.33	0	0 - 1
1C-value (pg)	3.52 ± 5.93	1.54	1.3	0.15 - 60.10
Ploidy level	3.12 ± 1.82	2	2	2 - 16
b) Diploid taxa, n=214	Mean ± stdev	Median	Mode	Range
Nitrogen	5.12 ± 1.75	5.5	6	1 - 9
Light	6.81 ± 1.25	7	7	3 - 9
Water	5.52 ± 1.86	5	5	2 - 12
Competition	0.35 ± 0.27	0.33	0	0 - 1
1C-value (pg)	2.97 ± 5.74	1.23	0.65	0.15 - 47.30
c) Polyploid taxa, n=142	Mean ± stdev	Median	Mode	Range
Nitrogen	5 ± 1.88	5	7	1 - 9
Light	6.87 ± 1.06	7	7	3 - 9
Water	5.8 ± 2.17	5	5	2 - 12
Competition	0.33 ± 0.27	0.29	0	0 - 1
1C-value (pg)	4.24 ± 5.07	2.34	0.55	0.30 - 30.19